STERILITY IN THE RABBIT ASSOCIATED WITH SOYBEAN HAY FEEDING[1]

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THREE FIGURES

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In the course of three experiments designed to test the effects of soil fertilization upon the nutritive value of feeds, as measured by lactation response in the rabbit (Kendall et al., '48), it became necessary, in the last two experiments, to change the roughage portion of the ration from lespedeza hay to soybean hay. When the change was made, so that soybean hay made up 49.5% by weight of the diet in place of lespedeza hay, a reduction in litter size in New Zealand white rabbits resulted. Abnormal reproduction in ewes fed soybean hay has been reported in two cases (Hunt, '35; North Carolina Agr. Exp. Sta. Bull., '44). The reasons for these failures are not known and, in our case, it was not at first clear that the smaller litter size was a consequence of the soybean hay intake.

It is the purpose of this paper to report the results of experiments designed to determine whether or not the reproductive failure could be related to soybean hay feeding and to describe the situation which obtained.

[1] An abstract of this paper was presented at the 45th annual meeting of the American Dairy Science Association, June, 1950, held at Cornell University, Ithaca, New York.

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EXPERIMENTAL

The experimental plans for the first three experiments have been reported previously (Kendall et al., '48). The ration consisted of 49.5% by weight of the particular field-cured hay used, 49.5% of ground wheat, and 1% of sodium chloride. In the first three experiments the general practice was to mate the females after they had been on the experimental diets for a preliminary period of two weeks. In the later trials the matings were made on the day the experimental feeding started. The females in the first three experiments were pair-fed; therefore, the intake of the diets was limited by the pair mate consuming the smaller amount. In the later experiments ad libitum consumption was allowed. As the soil treatments had no observable effect on the phenomenon reported here, no attempt will be made to report the evidence on that basis. The hay and other feeds used were seemingly of excellent quality. The lespedeza and soybean hays and the wheat were all grown on the same Soil Experiment Station field at Newton, Illinois, except in the cases indicated. The soybean hays grown on the Newton plots were all cut when the seeds were approximately one-half formed. The alfalfa hay, timothy hay, and corn used in the later experiments were purchased locally.

All of the males used in the test were highly fertile, as determined by control matings, and were fed a stock diet consisting of alfalfa hay and a commercial rabbit ration.

Similar procedures were followed in subsequent experiments except that detailed studies were not made of the carcasses of the offspring after a standard lactation period.

RESULTS

The numerical data for the first three experiments are given in table 1. In trial 1 with field-cured lespedeza hay, wheat and sodium chloride, the 12 females mated without difficulty. Twelve litters were kindled and ranged in size from 5 to 13 young. The average litter size was 8.4 young per litter.

Eleven of the 12 litters standardized for number of young survived the 15-day lactation period. In trial 2 (soybean hay + wheat) the females were placed with the male at the end of the preliminary feeding period. Only 5 of the 13 females mated at that time. The remaining 8 females were placed with the male daily for periods ranging from 19 to 27 days without mating normally. It then was necessary to hold the females to permit mating. Of the 8 females that were "force-mated," 4 kindled litters ranging in size from 4 to 7 young per litter. The remaining 4 "force-mated" females failed to show the characteristic gains in body weight which

TABLE 1

A comparison of body weights, feed intakes and litter sizes of females fed lespedeza and soybean hays

TRIAL NO.	RATION	NUMBER OF FEMALES		BODY WEIGHTS OF FEMALES		FOOD INTAKE DURING GESTATION	AVERAGE LITTER SIZ
		Mated	Producing litters	At mating	After parturition		
				kg	*kg*	*kg*	*no.*
1	Lespedeza hay + wheat	12	11	4.0	3.9	3.5	8.4
2	Soybean hay + wheat	13	9	4.1	4.0	3.2	6.3
3	Soybean hay + wheat	10	6	3.9	3.9	3.7	5.2

normally occur during gestation. Whether they ever became pregnant is not known. The average litter size for the 9 litters born was 6.3 young per litter. Three of the 4 litters resulting from "forced mating" failed to survive longer than 7 days. The mothers neither plucked hair for nests nor nursed their young satisfactorily.

Trial 3 was essentially a repetition of trial 2. However, in this and subsequent experiments the females were mated the day they were placed on the experimental diets. Ten matings produced 6 litters ranging in size from three to 9 young. The average litter size was 5.2 young per litter.

In all three of these trials the feed intakes of the females were fairly comparable. The average weights of the females at mating and immediately after parturition showed but slight variation. These facts, as shown in table 2, indicated that the lespedeza hay-wheat ration and the soybean hay-wheat ration were comparable in their ability to supply nutrients

TABLE 2

A summary of the reproductive performance of females as related to the ration fed

TRIAL NO.	TRIAL RATION	FEMALES MATED	KNOWN PREGNANCIES	LITTERS BORN AT TIME OF PARTURITION	YOUNG BORN	MEAN LITTER SIZE	INDIVIDUALS THAT HEMORRHAGED
1	Lespedeza + wheat	12	12	12	101	8.4	0
Control	Alfalfa + commercial ration	14	13	13	111	8.5	0
8	Alfalfa hay + wheat	4	4	4	32	8.0	0
7	Timothy hay + corn	4	3	3	23	7.7	0
	Total	34	32	32	267	8.3	0
2	Soybean hay + wheat	13	9	9	56	6.3	Not observed during pregnancy
3	Soybean hay + wheat	10	6	6	32	5.2	Not observed during pregnancy
4	Soybean hay + wheat	24	16	13	68	5.2	12
5	Soybean hay + wheat	9	7	7	48	6.9	2
6	Soybean hay + wheat	15	10	4	22	5.5	8
	Total	71	48	39	226	5.8	22

for maintaining body weight in the females. Thus the effect noted probably was not due to reduced caloric intake. In respect to total protein and to total mineral intake the rations were similar, the soybean hay diets being slightly higher in content of protein and minerals.

In the first experiment the primary objective was to secure large litters. Because the females refused to mate and litter sizes were relatively small when the soybean hay-wheat diet was fed, attention was focused on the problem under discussion.

The results of the first three and the following experiments are summarized in table 2.

Trials 4 and 5 were conducted in order that a more critical study of the females might be made during the 31-day period following mating. Feeds from the same fields were used, but in these trials the hay was only partially cured in the field and curing was completed in a mow having forced ventilation. In trial 4 (soybean hay + wheat), each of the 24 females mated almost immediately when placed with the male. Sixteen of the 24 matings resulted in known pregnancies, as indicated by the body weight increases of the females following mating. One female died 13 days after mating. There was no evidence to indicate that this female had been pregnant. Two other females died, both late in the gestation period. One other female, because of severe hemorrhage from the urogenital tract, was sacrificed at about the time for parturition. In all three of these animals extravasation of blood, emanating in one from the site of placentation and from the uterine wall in the other two, was evident at autopsy. Fetuses in advanced stages of resorption were present in all three. Plate cultures failed to reveal the presence of any pathogenic organism.

Hemorrhage, observed as blood flowing from the vagina and varying in severity, occurred in trial 4 in 12 females, during the latter half of the gestation period or at parturition. Four of these 12 females failed to bear young. Eleven of the 12 cases of hemorrhage occurred at times varying from two to 12 days prior to the expected date of parturition. The

voiding of partially resorbed fetuses was not uncommon where hemorrhaging occurred. In several instances ante mortem blood samples failed to clot normally. Out of the 16 known pregnancies, 13 females kindled living young with litters ranging in size from two to 12, with an average litter size of 5.2 young. One litter of three, though carried to the time for parturition, died shortly after parturition. These young were abnormal in that gross hemorrhage was observed around the ears of one and internal hemorrhage had occurred in the other two, the most pronounced hemorrhage occurring

Fig. 1 This rabbit was from a litter of 4 born on the 35th day of gestation. The hair had grown to a length of 2 mm.

within the pericardial sac. Two other litters were kindled two to 4 days after the end of the normal 31-day gestation period. The young in these litters were covered with hair 1 to 2 mm in length, as is shown in figure 1. The teeth of these young were approximately twice the size of those of young rabbits born at the end of a normal gestation period.

In trial 5 (soybean hay + wheat), 9 of the matings resulted in 7 litters kindled, ranging in size from 4 to 9 young and averaging 6.9 young per litter. Only two cases of severe hemorrhage occurred. In 5 of the 7 litters born from one to 6

young were stillborn. Of the 48 young born in the 7 litters, 14 were stillborn.

Trial 6 (soybean hay + wheat) was conducted with soybean hay grown on the University farm on soils of high fertility and cut before the seeds were formed. This hay was cured in a ventilated mow. The results of trial 6 were similar to those of trial 4. There were 15 known matings. Eight of the 15 females hemorrhaged, with varying degrees of severity. Twenty-five days after mating one female died following partial paralysis of the hind legs. There was no evidence that this female had been pregnant. Lesions in the gastric mucosa were first observed in this female. Congestion and diffuse hemorrhage in the cervix were also present.

In all, 7 of the females died, three of which were carrying fetuses. These three females showed varying degrees of uterine morbidity. In the most severe case the entire uterus was found to be very dark in color and friable, and had become gangrenous. The extravasation of blood was evident. Four partially resorbed fetuses were present. Lesions appearing to be focal hemorrhages were found in the gastric mucosa; a typical case is shown in figure 2. In one female, sacrificed after severe hemorrhaging on the 21st day following mating, 7 fetuses were found, of which 4 were alive and three were dead. The very dark colored, friable, right horn of the uterus, which contained the three dead and partially resorbed fetuses, is clearly shown in figure 3. This was typical of the cases observed of pregnant females that died in both trials 4 and 6. In two other females, similar to the one just described, with uterine bleeding and gastric lesions (see fig. 2), free blood was found in the thorax, due to what appeared to be a rupture of one of the larger blood vessels. In trial 6 there were 10 known pregnancies, 8 of the females hemorrhaging. Four litters were carried to the time of parturition, averaging 5.5 young per litter.

The failure of the blood to coagulate normally in those individuals which died or were sacrificed was not due to a prolonged prothrombin time. Average prothrombin times

(Quick and Leu, '37) of 10 to 14 seconds were observed after introducing thromboplastin into the oxalated plasma–$CaCl_2$ system. Qualitative tests failed to show the presence of heavy metals in the tissues of the animals that died.

The observations made during experiments 4, 5, and 6 indicated that the small litters observed earlier in experiments 2 and 3 may have been due to the failure of the females to carry all implantations to the time of parturition. In these later

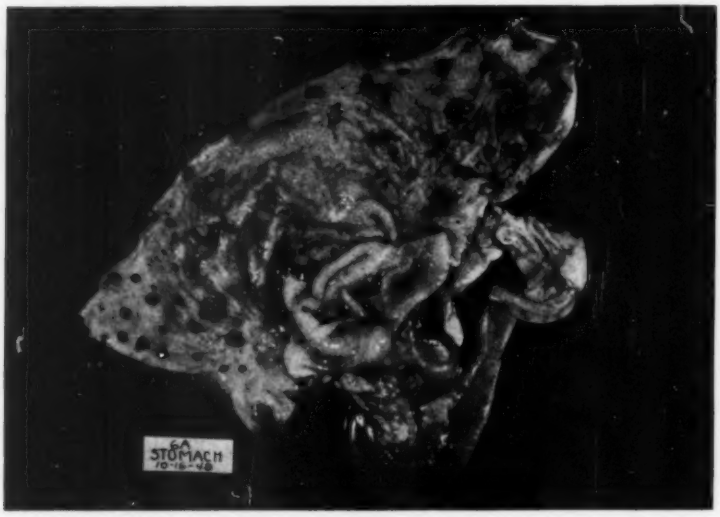

Fig. 2 The gastric mucosa in this stomach contained numerous lesions 1 to 2 mm in diameter.

trials uterine hemorrhage, fetal resorption, and general hemorrhage were observed, apparently the results of endothelial breakdown. The failure of the blood to clot normally was seemingly not due to an effect upon prothrombin. It appears that the effects observed were not due to the soil treatments or lack of them with respect to the soybean hay produced for trials 2, 3, 4, and 5. Soybean hay produced on a highly fertile soil and fed in trial 6, in combination with the

Fig. 3 The dark, hemorrhaged right uterine horn contained three partially resorbed fetuses. Blood was flowing from the urogenital tract before the animal was sacrificed.

wheat grown on the experimental soil plots at Newton, Illinois, gave the same results. Also the results were probably not due to the stage of maturity at which the soybean plants were cut for hay, as the early-cut hay used in trial 6 gave results similar to those observed with the later-cut hays used in trials 2, 3, 4, and 5. The symptoms observed in trial 6 were not caused by bacterial infection.

It remained to demonstrate whether or not the syndrome was the consequence of a lack of some essential nutrient or nutrients. These experiments are still in progress. However, the results of two experimental trials and a control study are now available. In the first instance (trial 7) a ration considered to be inferior to the earlier diets used was fed to 4 similar females. The ration was made up of poor-quality timothy hay, corn grain, and sodium chloride in the same proportions as the hay, wheat, and salt which were fed in the other experiments. Because of the timothy hay, this ration was lower in nutrients than the other rations used. The 4 females mated without difficulty. Three of the 4 kindled litters ranging in size from 4 to 10 young, with an average of 7.7 young per litter. No hemorrhages of the females nor stillbirths were noted. The young survived and grew normally while suckling the females fed the timothy hay-corn diet.

In trial 8 alfalfa hay obtained locally was fed in the same proportion with salt and with wheat grown on the soil experimental plots at Newton, Illinois. The 4 females all conceived and produced an average of 8 young per litter, the number of young varying from 6 to 10. No hemorrhages or stillbirths resulted. The young in these litters survived and grew normally. This trial suggested that the wheat, at least when in combination with alfalfa, was not a suspect.

On the control stock diet of alfalfa hay and a commercial rabbit mixture, 14 females produced 13 litters ranging in size from 4 to 13 young, with an average of 8.5 young per litter.

DISCUSSION

It appears that the partial failue in reproduction observed in these studies is associated with the feeding of soybean hay.

Wheat, it would seem, is not a factor, since the feeding of wheat with lespedeza hay or alfalfa hay resulted in normal pregnancy, normal litter size, and no indication of impaired body functions.

The specific factor or factors responsible for the syndrome are not evident at the present time; however, there are a number of questions that might be raised. Could the adequacy of the protein have been a factor, even though the total nitrogen content of the soybean hay was higher than that of any of the roughages fed? Growth studies have shown that rations containing raw soybeans were improved by the addition of cystine or methionine (Hayward and Hafner, '41; Evans and McGinnis, '46; Borchers et al., '47), while in other studies a depressed growth response was believed to be due to an inhibition of the proteolytic activity of trypsin by a trypsin inhibitor in the raw soybean (Ham et al., '45). Could a trypsin inhibitor have had some influence upon the impaired body functions observed in these studies?

That a ration consisting of approximately one-half wheat grain should supply adequate tocopherol to meet the animals' needs is fully recognized. Similarly, high-quality legume roughage should supply adequate provitamin A. Nevertheless, many of the observed clinical symptoms—namely, foetal hemorrhage, vascular breakdown in the thorax and uterus, mature female mortality, fetal death, fetal resorptions, stillbirths, some cases of prolonged gestation, mature female paralysis, normal prothrombin time, gastric lesions and focal and diffuse hemorrhage in various other parts of the body—resulting from the feeding of soybean hay to New Zealand white rabbits appear to be similar in many respects to many of the reported symptoms associated with vitamin A (Mason, '35; Jensen, '46) or vitamin E (Barrie, '38; Evans and Burr, '27) deficiency in the rat.

Because of the similarity between the symptoms described here with respect to the rabbit and those reported elsewhere, especially in relation to the vitamin A-deficient rat, the question is raised of whether or not vitamin A deficiency is a con-

tributing factor in the syndrome. If the syndrome is a consequence of inadequate levels of vitamin A, could such a deficiency exist in the soybean hay-fed rabbit because of carotene destruction due to a coupled oxidative reaction associated with the enzyme lipoxidase? Such destruction, believed to be due to enzymic action, has been shown to occur in the field-curing of alfalfa (Mitchell and Hauge, '46).

The presence of lipoxidase in the soybean seed (Andre and Hou, '32) is well-known. The existence of the enzyme has also been reported in the soybean plant in its earlier stages of development (Holman, '48). The abili'y of lipoxidase to peroxidize unsaturated fats has been ably demonstrated (Sumner and Dounce, '39; Strain, '41; Thorell et al., '46). The intermediate products of the reaction involving peroxidation of unsaturated fats by lipoxidase have a bleaching action upon carotene (Sumner and Dounce, '39). Carotene thus bleached has been shown to be ineffective as a source of provitamin A for promoting animal growth (Frey et al., '36). Could provitamin A destruction through the action of lipoxidase, whether it occurred in the curing of the hay or in the gastrointestinal tract, be an underlying factor contributing to the symptoms observed when New Zealand white rabbits were fed soybean hay as one-half of the diet?

Etiological studies made with a view to answering these questions are now in progress.

SUMMARY

Partial reproductive failure in the New Zealand white rabbit characterized possibly by a failure in implantation or early fetal death, decreased litter size, stillbirths, frequent vascular breakdown, particularly in the uterus, numerous partial fetal resorptions, and some partial paralysis of does has been observed as a consequence of soybean hay feeding. The data for does fed rations which contained roughages other than soybean hay included 34 mated females with 32 known pregnancies resulting and 32 litters born at the time

of parturition, totaling 267 young, with an average litter size of 8.3 young per litter. Data from studies where soybean hay was fed included 71 mated females, 48 known pregnancies, 22 cases of vascular breakdown, and 39 litters born at the time of parturition, totaling 226 young, with an average litter size of 5.8 young per litter. In some cases females on the soybean hay diet refused to accept the male when the diet was fed prior to the date of mating; others lost their litters from failure to provide nests and to suckle the young satisfactorily; and still others died during gestation, following severe hemorrhage.

Research leading to identification of the specific factor or factors contributing to the impaired reproduction in the New Zealand white rabbit fed soybean hay is indicated.

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ARTIFICIAL ENRICHMENT OF WHITE RICE AS A SOLUTION TO ENDEMIC BERIBERI

REPORT OF FIELD TRIALS IN BATAAN, PHILIPPINES

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THREE FIGURES

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This is the third paper of a series. In the first paper (Salcedo et al., '48) detailed results were given of a clinical survey undertaken during the period July 1, 1947, to February, 1948, of the incidence of beriberi in Bataan. In brief, 12,384 persons taken at random out of a total estimated population of 92,162 as of October 1, 1947 [1] were individually examined. Of those examined, 245 showed frank beriberi, 443 "suggestive" beriberi and 892 "doubtful" beriberi, a total of 1,580 cases with one or more indicative symptoms. It thus appeared that an average of 12.76% of the population was more or less affected by the disease. These findings are consistent with the known mortality from beriberi, both in Bataan and in the Philippines as a whole, through many years (see fig. 2). Beriberi has recently ranked second only to tuberculosis as a cause of death in the Philippines, the respective

[1] The population figures given in previous papers have been somewhat at variance with one another due to lack of an official census and disturbed post-war conditions. The figure given here is the most mature official estimate, based on the later census enumeration of October 1, 1948.

mortality rates per 100,000 having been, in 1947, 141.91 and 168.23.

The foregoing survey was undertaken in preparation for a large-scale experimental introduction of fortified rice. The second paper in the series (Salcedo et al., '49b) gives an account of the preliminary acceptability trials of the rice which was proposed for use, a statement concerning inauguration of the distribution of such rice in Bataan on October 1, 1948, and the figures on mortality from beriberi during the first three months after such inauguration. As compared with the corresponding quarter of the preceding year, deaths from beriberi had fallen in the enriched rice area from 45 to 20, while those in the remainder of the province had risen from 8 to 12.

In the present, third paper of the series, the results are given of a second clinical survey during the period July 1, 1949, to February 28, 1950, two years later than the first. Also later mortality figures, now available, are presented, together with a further account of the progress of the experiment and a discussion of the significance of the findings. An account of certain of the results obtained prior to July 1, 1949, has already appeared (Salcedo et al., '49a).

EXPERIMENTAL

Plan of the experiment

The Bataan experiment was conceived not merely as a physiological trial of enriched rice versus ordinary white rice such as might have been achieved under conditions of enforced consumption, as in an institution having inmates under discipline. It also contemplated appraisal of all the socio-economic factors which may influence the choice of dietary by a people living in their customary environment and buying their food in the open market, subject to the prejudices, persuasions and inhibitions to which custom or trade practices may subject them. To obtain significant results under such conditions it is essential that the population

shall be large enough to permit statistically valid conclusions, and that it constitute an independent economic society reasonably free from extraneous influences which would be incapable of proper observation and measurement. Hence the choice of the relatively isolated and economically homogeneous province of Bataan.

Comparisons were contemplated of the prevalence of beriberi in Bataan before and after introduction of the new rice, as well as with the prevalence in other provinces. However, it also seemed desirable to use a nearby but isolated population as a control. Accordingly, it was early decided to set up within Bataan a control area as well as an experimental one, which might then be compared with one another with respect to morbidity, mortality and other features before and after a reasonable trial period. The 7 municipalities of the east coast, with a population of 63,508 as of October 1, 1948, made up the experimental area (area A); the remainder of the province, comprising a larger, more sparsely settled area in 5 municipalities, with a population of 29,393, became the control zone (area B; see map, fig. 1). The boundaries of each zone were fixed with reference to those of the municipalities and also to the location of roads and rice mills, so as to minimize the hazards of rice being transported without our knowledge from the control zone to the experimental area, or its infiltration from other provinces. This subdivision proved successful on the whole, although there was infiltration of enriched rice into one municipality, Bagac, to the extent of 20 to 34% of the residences surveyed in the first half of 1949, as determined by systematic rice inspection (and perhaps infiltration elsewhere in varying degrees).

The clinical surveys of 1947 [2] and of 1949 [3] covered all 7 municipalities of the experimental zone. In the control zone, however, they were confined to the two municipalities of Bagac and Moron. This seemed necessary because of limitations of personnel, distances, availability of local health of-

[2] July 1, 1947, to February, 1948.
[3] July 1, 1949, to February 28, 1950.

ficers for assistance, and like considerations. Comparisons of mortality statistics, however, cover the entire populations of both control and experimental zones.

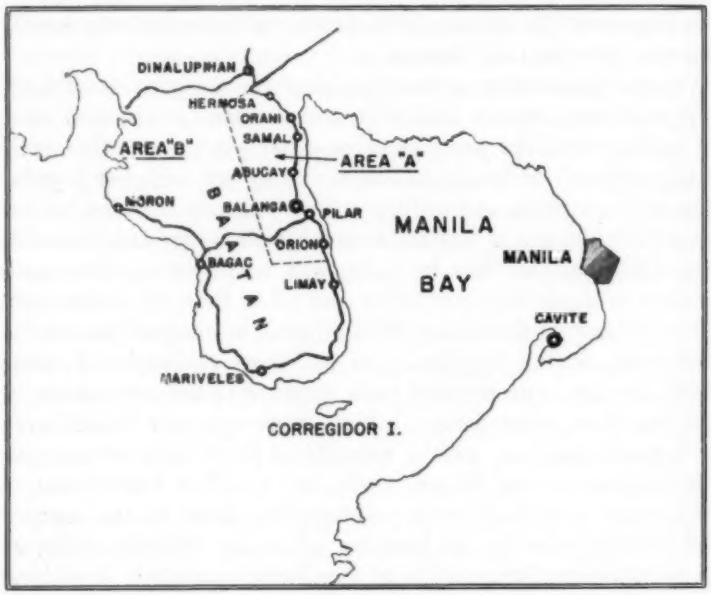


Fig. 1 Peninsula of Bataan and environs. The experimental area (area A) is outlined only approximately by a broken line. The remainder of the province, area B, used as a control area, is largely mountainous and much more sparsely settled.

The rice distribution system

The rice premix was manufactured in the United States substantially according to the method of Furter et al. ('46) and shipped to Bataan. (A premix plant has subsequently been erected in Manila by the United States Public Health Service and donated to the National Rice and Corn Corporation, a Philippine Government subsidiary. It is now producing premix for Bataan and for the further extension of the rice enrichment program.) Riboflavin was omitted lest the

yellow stained grains be discarded by housewives. Otherwise the premix was designed to produce enriched rice containing, per pound, 2 mg thiamine, 16 mg niacin and 13 mg iron, in accordance with the U. S. Federal standard for enriched flour and enriched corn meal. The premix was delivered to the mills of Bataan free of cost. (After October 1, 1950, the millers will be charged for it substantially at cost and will be authorized to charge the purchaser of enriched rice a premium of not more than 1.2 centavos per ganta — approximately 5 lb. This corresponds to 12 cents U. S. currency per 100 lb., or a per capita annual cost of not more than 35 cents based on a daily consumption of 360 gm of rice.)

Each of the 23 cone mills in the province was also supplied free of charge with mixing machinery (cost, $125 to $175 per mill, according to size) for blending the premix with all the white rice which they produced, in proportion of 1 lb. of premix to 199 lb. of rice. Several plantation hullers, of the Engelberg type, used as small production mills, were also provided with a single feeder at a cost of $35.00 per mill. No legal compulsions were resorted to but the compliance of the millers was secured by an appeal for cooperation and in consideration of the issue without cost of the premix and the mixing machinery.

The rice inspection system

Inspectors were employed and trained to examine the enriched rice twice weekly at each mill, once a week in markets and rice shops and occasionally in homes. Nearly all homes were inspected at least once during the first year. The method was extremely simple; namely, to spread a standard cup of rice (375 ml) in a thin layer and count the premix grains. Standard counts were fixed: 60 per cup for large grained rices, 90 for smaller grained varieties. The premix grains can be detected by visual inspection after a little training and it was rarely necessary to resort to staining with thiocyanate (iron in premix coating) or with iodine, which fails

to blue the coated premix grains. Although there was occasional infraction of the rules within the experimental zone, indicating dilution with unenriched rice or excessive fortification with premix, deviation from the norm involved not more than 10% of the total rice supply.

Much more serious from an experimental viewpoint was the infiltration of enriched rice into the control area. Inspection in Bagac in the early part of 1949 showed some enriched rice in 20 to 34% of the homes. Similar infiltration probably occurred in Limay, which normally gets about 30% of its rice from Balanga and Pilar. In more remote Moron only 1% of the homes had enriched rice at this time. By September, 1949, it became impossible because of public demand to maintain exclusion of enriched rice from the control area, and enriched rice was deliberately introduced into Bagac and Moron through their rice mills on October 1, 1949. These facts impair considerably the comparisons between experimental and control zones, as will be noted later.

It should be borne in mind that it was necessary to permit the millers to continue to operate on business principles, milling rice for a fee for farmers, buying rough rice (paddy) from whom they chose, and selling the milled product through their accustomed channels. Otherwise rice distribution would have taken on a compulsory character and the significance of the experiment would have been lost with respect to socio-economic factors. Some infiltration was perhaps inevitable under the circumstances.

The second clinical survey

The organization of this survey, the clinical methods used and the modes of compiling statistics followed those of the previous survey and have been discussed in an earlier paper (Salcedo et al., '48). The numbers and geographical distribution of the persons examined were very similar, including the 7 municipalities of the experimental area and the two municipalities of the control region. Both surveys began

July 1 in the respective years and in both areas simultaneously. The total number of persons examined in the present experiment was 11,492, of whom 10,877 were individuals who had been examined during the survey two years earlier. Table 1 shows the distribution of the persons examined, by municipality and classification.

Table 2 gives the incidence of beriberi found in each municipality, classified by degree of severity and type of

TABLE 1

Breakdown of number of individuals examined by municipality and population group in 1949 clinical beriberi survey

ENRICHED RICE OR EXPERIMENTAL AREA	INFANTS	CHILDREN (2.01–15 yrs.)	EXPECTANT MOTHERS	NURSING MOTHERS	OTHER ADULTS	TOTAL
Hermosa	0	379	47	111	393	930
Orani	27	514	158	331	622	1,652
Samal	16	358	82	204	707	1,367
Abucay	1	345	67	176	684	1,273
Balanga	0	376	44	119	724	1,263
Pilar	0	401	59	216	655	1,331
Orion	0	330	72	192	702	1,296
Total	44	2,703	529	1,349	4,487	9,112
CONTROL AREA OR NON-ENRICHED AREA						
Bagac	0	525	32	76	511	1,144
Moron	2	315	40	149	730	1,236
Total	2	840	72	225	1,241	2,380
Grand total	46	3,543	601	1,574	5,728	11,492

person. It will be noted that the total incidence in the experimental area was 1.55% as compared with 8.7% in the control area, and also that in the latter area the incidence in Bagac was less than one-third that in Moron; due, it is believed, to infiltration of enriched rice into the former municipality as previously noted. Table 3 compares the in-

cidence in each municipality in the two successive surveys. It is evident that nearly 90% of the clinical beriberi had disappeared in the experimental area. In the control area beriberi was somewhat diminished in Bagac but much increased in Moron. The reason for this increase is not definitely known.

TABLE 2

Incidence of clinical beriberi in Bataan by degree of deficiency and place of residence, 1949–1950 survey

MUNICIPALITY	FRANK BERIBERI	SUGGESTIVE BERIBERI	DOUBTFUL BERIBERI	TOTAL	
	Cases	Cases	Cases	Cases	Incidence
	no.	no.	no.	no.	%
Experimental area:[1]					
Hermosa (11%)	1	8	6	15	1.61
Orani (12%)	0	3	8	11	0.67
Samal (20%)	0	2	5	7	0.51
Abucay (13%)	2	17	8	27	2.12
Balanga (10%)	1	3	20	24	1.90
Pilar (23%)	1	3	4	8	0.60
Orion (15%)	2	16	31	49	3.78
Total	7	52	82	141[2]	1.55
Control area:					
Bagac (42%)	14	16	17	47	4.11
Moron (28%)	69	63	28	160	12.94
Total	83	79	45	207[3]	8.70
Grand total — 9 out of 12 towns	90	131	127	348	3.03

[1] Numbers enclosed by parentheses indicate per cent of population examined.
[2] Two children, 37 expectant mothers, 42 nursing mothers, 60 other adults.
[3] Two children, 21 expectant mothers, 71 nursing mothers, 113 other adults.

It is also of interest to note the changes in the severity of the symptoms of those individuals who were examined in both of the successive surveys. These are shown in table 4. It is clear that the severity diminished greatly in the experimental area but often increased in the control zone. The number of individuals in the control zone who were symptom-free in 1947 but had symptoms in 1949 is further evidence

TABLE 3

Influence of enriched rice on incidence of clinical beriberi in Bataan classified as to degree of severity and place of residence
Note: First survey (1947) made before enrichment; second survey (1949) made after enrichment)

MUNICIPALITY	FRANK BERIBERI INCIDENCE			SUGGESTIVE BERIBERI INCIDENCE			DOUBTFUL BERIBERI INCIDENCE			TOTAL BERIBERI INCIDENCE		
	1st Survey	2nd Survey	Reduction	1st Survey	2nd Survey	Reduction	1st Survey	2nd Survey	Reduction	1st Survey	2nd Survey	Reduction
	(a)[1] %	(b) %	(c) %	(a) %	(b) %	(c) %	(a) %	(b) %	(c) %	(a) %	(b) %	(c) %
Experimental or enriched rice area:												
1. Hermosa	1.15	0.11	90.69	2.46	0.86	65.04	9.61	0.65	93.28	13.23	1.61	87.81
2. Orani	0.94	0.00	100.00	1.53	0.18	88.16	6.94	0.48	93.02	9.41	0.67	92.93
3. Samal	0.37	0.00	100.00	1.55	0.15	90.58	7.32	0.37	95.01	9.18	0.51	94.42
4. Abucay	1.53	0.16	89.73	3.59	1.34	62.81	6.19	0.63	89.85	11.30	2.12	81.23
5. Balanga	1.52	0.08	94.18	7.93	0.24	97.08	14.75	1.58	89.27	24.26	1.90	92.17
6. Pilar	2.18	0.08	96.56	3.96	0.23	94.32	4.55	0.30	93.40	10.69	0.60	94.38
7. Orion	5.92	0.15	97.39	6.34	1.23	80.53	3.70	2.39	35.37	15.96	3.78	76.31
Total	2.07	0.10	96.28	4.17	0.57	86.33	8.06	0.90	88.85	14.30	1.55	89.18
Control area:												
1. Bagac	2.86	1.22	57.23	1.47	1.40	4.89	1.39	1.49	+ 6.91	5.72	4.11	28.18
2. Moron	0.16	5.65	+ 3,417.50	0.81	5.10	+ 529.26	5.92	2.27	61.74	6.73	12.94	+ 92.33
Total	1.62	3.49	+ 115.24	1.21	3.32	+ 174.29	3.83	1.89	50.65	6.66	8.70	+ 30.59
Grand total (9 out of 12 municipalities)	1.99	0.78	60.66	3.58	1.14	68.18	7.24	1.11	84.74	12.76	3.03	76.27

[1] (a) From column (b), table 2, J. Nutrition, *36:* 566, Nov., 1948.

(c) $\frac{(a) - (b)}{(a)} \times 100\%$.

(+) Means increase in incidence.

TABLE 4

Changes in severity of symptoms in individuals examined in 1947 and re-examined in 1949

BERIBERI TYPE	CASES 1947	CASES RE-EXAMINED 1949	NO CHANGE	BECAME FRANK	BECAME SUGGESTIVE	BECAME DOUBTFUL	NO SYMPTOMS	SYMPTOM FREE	NEW CASES 1949	
									Symptom free in 1947	Not examined in 1947
								%		
Area A										
Frank	206	144	7	..	15	16	106	73	0	0
Suggestive	414	362	18	0	..	26	318	88	0	3
Doubtful	800	593	34	0	16	..	543	89	0	6
Total	1,420	1,099	59	0	31	42	967	88	0	9
Area B										
Frank	39	31	11	..	7	4	9	29	30	14
Suggestive	29	20	9	4	..	6	1	5	26	14
Doubtful	92	78	14	22	23	..	19	24	16	3
Total	160	129	34	26	30	10	29	22	72	31

of the validity of the observed increase in incidence in Moron, as noted in table 3.

Mortality

Figures on deaths from beriberi were collected in the Philippines from 1924 until the Japanese occupation late in 1941. Collection of the figures was resumed in 1946. The rates for Bataan Province and for the Philippines as a whole are indicated in figure 2.

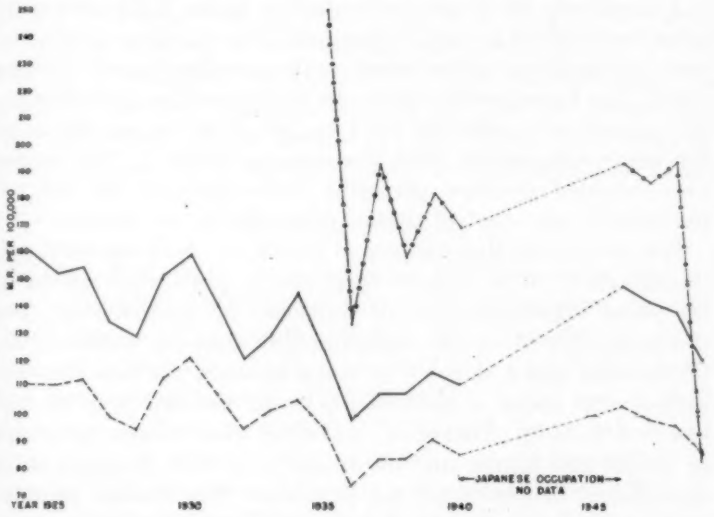


Fig. 2 Mortality rates from beriberi per 100,000 population in the Philippines and in Bataan 1924–1940 and 1946–1949. Statistics from the Bureau of Health, Manila. The statistical year is July 1–June 30 and is designated by the later calendar year. Due to destruction of records by war, few pertinent data can be found for beriberi mortality in Bataan in earlier years. However, rates comparable to later pre-war years are indicated, e.g., 200 infant beriberi deaths were reported from Bataan in 1932.

———— Total beriberi mortality, Philippines.

– – – – – – Infant beriberi mortality, Philippines.

–o–o–o–o–o– Total beriberi mortality, Bataan.

In Bataan, under the direction of the district health officer, Dr. Francisco Hernando, who was succeeded in 1948 by Dr. M. D. Bamba, special care was given to the collection of these mortality statistics, beginning in June, 1947. All government physicians in the Province, including the medical teams who conducted the clinical surveys, assisted in this task. As far as possible all diagnoses of beriberi were confirmed by consultation. Also the mortality records were compiled quarterly instead of annually, as had been the standard practice in pre-war years.

Accordingly there are presented in figure 3 the mortality rates for beriberi in each municipality for 12 successive quarters, 5 preceding and 7 succeeding the introduction of enriched rice in the experimental area. In each case the mortality for the quarter is multiplied by 4 to put it on an annual basis for ready comparison with the data in figure 2. Curves are also included to show mortality from beriberi for the experimental and control areas, respectively, as wholes.

Because of the fluctuations in mortality, both seasonal and "accidental," it is difficult to choose a particular period as the most significant for all purposes of comparison. The downward trend in the experimental area is, however, unmistakable, and a contrast in trend between the experimental and control areas is indicated between October 1, 1948, and October 1, 1949. Thereafter enriched rice became available in Bagac and Moron and on January 1, 1950, it began to be distributed to nearly all the province. The decline in mortality in the control area late in 1949 and 1950 must be attributed to this cause.

Perhaps the most significant time comparison which can be made at present is the mortality from beriberi in the experimental area for the last full year before enrichment (July 1, 1947, to June 30, 1948) with that for the first corresponding period during which enrichment was in force (July 1, 1949, to June 30, 1950) in the experimental area throughout the year. The respective annual mortality rates per 100,000 for these two periods were 263.57 and 28.17. These figures may be

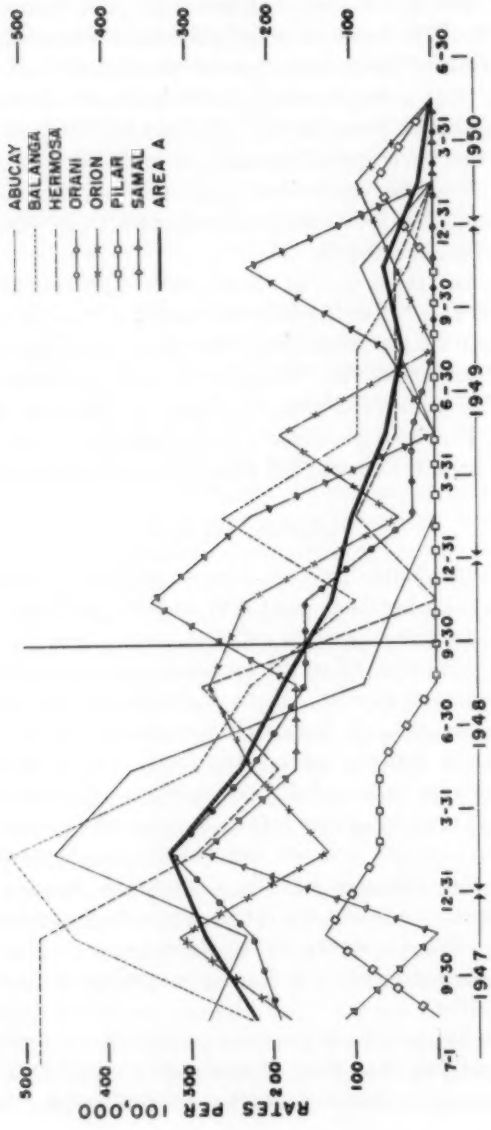
compared with those for all Bataan in past years as shown in figure 2. The beriberi mortality rate has dropped to a small fraction of that of the lowest previously recorded year in Bataan. Not a single death from beriberi occurred in the experimental area from April 1 to June 30, 1950, as compared with 37 in the corresponding quarter of 1948. Similarly, only 18 deaths from beriberi were reported in the year July 1, 1949, to June 30, 1950, while the deaths from this cause in 1947–1948 numbered 167.

Perhaps the most useful comparison between area A and area B with respect to beriberi mortality is that of the respective drops in death rates from the year preceding the introduction of enriched rice (October 1, 1947, to September 30, 1948) to the year following (October 1, 1948, to September 30, 1949). The rate for area A fell from 254 to 80, a drop of 68.5%. In area B the rate fell from 152 to 149, a drop of 1.5%.

Infantile beriberi

Since infant deaths have so long constituted a high proportion of the total beriberi mortality of the Philippines, usually two-thirds to three-fourths of the total (see fig. 2), it is imperative that the effect of enriched rice on infantile beriberi be inspected closely. Unfortunately, in the two clinical surveys in Bataan, as indicated in table 1, an unduly small proportion of infants were examined. It is impossible to examine infants successfully for milder symptoms. This is due to the character of the infantile form of the disease. Premonitory symptoms are not detectable by physical observation until the infant's life is already in danger, and the disease usually runs out its fatal course in two or three days thereafter. The incidence of beriberi among infants, as reported, is accordingly very low as compared with the known high mortality.

Accordingly, we are dependent primarily on mortality statistics in judging the effect of enriched rice on infantile beriberi. Figures on infant deaths from beriberi are given


ABUCAY
BALANGA
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ORANI
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SAMAL
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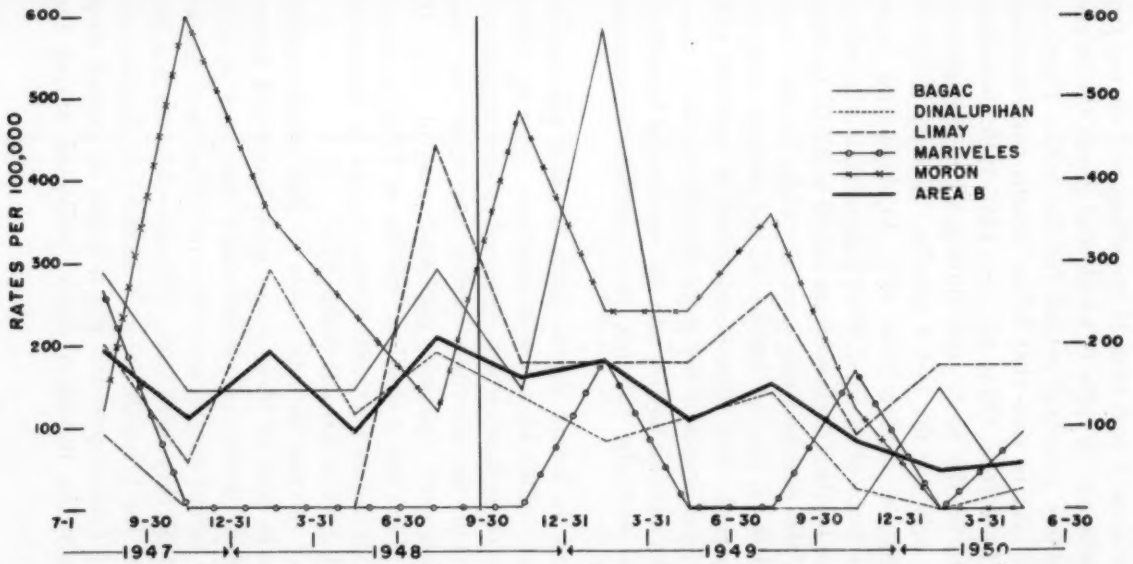

Fig. 3 Beriberi death rates per 100,000 population for every municipality of Bataan — July 1, 1947 to June 30, 1950. Enriched rice was introduced in area A on October 1, 1948. Quarterly death rates are computed to an annual basis and plotted at the midpoint of each quarter. The populations of the several towns as of October 1, 1948 were as follows: *Area A:* Abucay — 8,453, Balonga — 12,379, Hermosa — 8,437, Orani — 12,718, Orion — 8,721, Pilar — 5,805, Samal — 6,995. Total — 63,508; *area B:* Bagac — 2,745, Dinalupihan — 14,341, Limay — 4,509, Mariveles — 4,462, Moron — 3,336. Total — 29,393. One or two deaths in the smaller towns produce large changes in rate.

separately for area A and area B preceding and during the period of experiment in table 6, which also shows the infant deaths from three principal causes for the entire Province of Bataan. It may be seen that infant deaths from beriberi in area A declined from 143 in the year 1947–1948, preceding enrichment, to 68 the next year, during which enrichment was in effect only for the last 9 months. In area B infant beriberi deaths rose slightly from 42 to 45, during the same period. Or, in terms of rate per 100,000 population, the infant mortality from beriberi declined from 225.65 to 107.03 (a drop of 52.6%) in the enriched rice area, A, while it rose from 145.82 to 153.09 (a rise of 5.02%) in the control area, B, during this period. The pronounced effect of enriched rice on infant beriberi deaths is the more remarkable since these figures concern nursing babies, who ate no rice and benefitted chiefly, if not entirely, through their mothers' mammary glands.

The decrease in infant deaths from beriberi was unfortunately largely offset by a rise in mortality from an infectious enteritis (diarrhea), which appeared in severe epidemic form throughout the province during 1948–1949 but less acutely in the more sparsely populated control area, B. Nevertheless there was a small net decline of 2.6% in infant deaths from all causes per 1,000 births in area A, compared with a rise of 3.3% in area B (table 5).

It is also of interest to note (table 6) that infant deaths from beriberi in each of these years were many-fold larger than all beriberi deaths of people above one year of age. In 1947–1948 infant deaths were 88% of all beriberi deaths and in 1948–1949 they were 93%. This serves to confirm the prominence of the infantile form of beriberi in Bataan and to correct the false impression which might be gained from inspecting only the incidence figures collected during the surveys.

TABLE 5

Changes in infant mortality, crude mortality, tuberculosis mortality and beriberi mortality rates per 100,000 population in Bataan province during 1947–1949

YEAR	EXPERIMENTAL OR ENRICHED AREA Pop. = 63,508 [1] = 63,359 [2]					CONTROL OR NON-ENRICHED AREA Pop. = 29,393 [1] = 28,803 [2]					TOTAL FOR ALL BATAAN Pop. = 92,901 [1] = 92,162 [2]			
	Births	Deaths below 1 yr.	Total deaths	Deaths due to T.B.	Beri-beri deaths	Births	Deaths below 1 yr.	Total deaths	Deaths due to T.B.	Beri-beri deaths	Deaths below 1 yr.	Total deaths	Deaths due to T.B.	Beri-beri deaths
1947–1948	2,921	359	968	194	167	1,230	154	422	73	43	513	1,390	267	210
1948–1949	3,024	362	951	164	74	1,361	176	493	78	48	538	1,444	242	122
Difference		+ 3	— 17	— 30	— 93		+ 22	+ 71	+ 5	+ 5	+ 25	+ 54	— 25	— 88
Change in per cent		+ 0.83	— 1.75	— 15.46	— 55.7		+ 14.29	+ 16.8	+ 6.84	+ 11.6	+ 4.87	+ 3.88	— 9.36	— 41.90
Deaths/ 100,000 population (mortality rates)														
1947–1948		122.9 [3]	1,527.50	306.13	266.68		125.2 [3]	1,465.18	253.45	149.29	123.6 [3]	1,508.15	289.69	227.85
1948–1949		119.7 [3]	1,496.87	258.34	116.70		129.3 [3]	1,677.19	265.36	163.30	122.7 [3]	1,453.74	260.39	131.32

[1] As of October 1, 1948.

[2] As of October 1, 1947.

[3] Rate per 1,000 births.

TABLE 6

Deaths and mortality rates per 100,000 population from three causes of infant deaths, July 1, 1947 to June 30, 1949

(A = enriched rice area; B = control area; C = entire province)

AREA		DEATHS Under 1 yr.		DEATHS Above 1 yr.		M. R. Under 1 yr.		M. R. Above 1 yr.	
		'47–'48	'48–'49	'47–'48	'48–'49	'47–'48	'48–'49	'47–'48	'48–'49
Beriberi	A	143	68	24	6	226	107	38	9
	B	42	45	1	3	146	153	3	10
	C	185	113	25	9	201	122	31	13
Diarrhea	A	25	54	34	58	39	85	54	91
	B	15	19	13	20	52	65	45	68
	C	40	73	47	78	43	79	51	83
Broncho-pneumonia	A	25	28	29	31	39	44	46	49
	B	12	16	32	42	42	54	111	143
	C	37	44	61	73	40	47	66	79

Over-all mortality and tuberculosis

In table 7 are given the mortality rates for each of the 10 leading diseases, and also the total mortality in the two areas separately for two successive years. Since beriberi caused 10 to 17% of all deaths prior to the inauguration of the experiment, it would be expected that reduction of deaths from beriberi would have a discernibly favorable effect upon total deaths. Such is found to be the case, to some extent (see also table 5). Deaths from all causes declined in area A by 17 (1.8%), while in area B there was an increase of 71 deaths (16.8%), chiefly due to bronchopneumonia and diarrhea, which diseases also appeared in area A in epidemic form.

Tuberculosis is a disease in which good nutrition is believed to have a favorable influence. Accordingly, rice enrichment might conceivably reduce mortality from this cause. That this is so is indicated, but scarcely proved, by the figures in tables 5 and 7. Tuberculosis mortality declined in area A by 15%, while it rose in area B by 7%.

TABLE 7

Annual mortality rates of first 10 leading diseases in Bataan (by areas) for July 1, 1947 – June 30, 1949

JULY 1, 1947 – JUNE 30, 1948			JULY 1, 1948 – JUNE 30, 1949		
Diseases	Total deaths	M. R. per 100,000 pop.	Diseases	Total deaths	M. R. per 100,000 pop.
Enriched rice — Area "A"					
1. Tuberculosis	194	306.13	1. Tuberculosis	164	258.34
2. Beriberi	167	263.57	2. Diarrhea	112	176.28
3. Diarrhea	59	93.10	3. Beriberi	74	116.70
4. Bronchopneumonia	54	85.21	4. Bronchopneumonia	59	92.86
5. Acute bronchitis	23	36.29	5. Acute bronchitis	22	34.63
6. Pneumonia	16	25.25	6. (a) Malaria	15	23.61
7. Malaria	15	23.67	6. (b) Pneumonia	15	23.61
8. Influenza	12	18.93	8. Tetanus	10	15.74
9. Tetanus	3	4.74	9. Influenza	8	12.59
10. Whooping cough	2	3.16	10. Whooping cough	1	1.57
11. All causes	968	1,527.50	11. All causes	951	1,496.87
Control — non-enriched — Area "B"					
1. Tuberculosis	73	253.45	1. Tuberculosis	78	265.36
2. Bronchopneumonia	44	152.76	2. Bronchopneumonia	58	197.31
3. Beriberi	43	149.29	3. Beriberi	48	163.30
4. Diarrhea	28	97.22	4. Diarrhea	39	132.68
5. Pneumonia	22	76.38	5. Malaria	22	74.85
6. Malaria	15	52.08	6. Pneumonia	12	40.82
7. Acute bronchitis	10	34.72	7. Acute bronchitis	9	30.62
8. Tetanus	9	31.25	8. Influenza	5	17.01
9. Influenza	7	24.30	9. Tetanus	2	6.80
10. Whooping cough	0	0.00	10. Whooping cough	0	0.00
11. All causes	422	1,465.18	11. All causes	493	1,677.19

It should be borne in mind that enrichment was in effect in area A only during the last three-quarters of 1948–1949. Had it been possible to inaugurate enrichment three months earlier, as originally planned, this comparison could possibly have been more significant and perhaps more striking. The delay was due to slow deliveries of mixing machinery from the United States and was beyond our control. Mortality figures other than for beriberi (total) are not available by quarters, so adjustment to fit the enrichment program calendar is impossible. The statistics of future years will perhaps permit more conclusive comparison with pre-war years in Bataan, assuming that enrichment is maintained henceforth in accordance with the present intention. Fruitful comparisons with the statistics of other provinces may also be possible.

Unfortunately, future significant comparisons between areas A and B will be impossible because of the infiltration of enriched rice into area B, followed by deliberate distribution of it there subsequent to October 1, 1949. This represents one of the insurmountable difficulties of dealing with substantial human populations not subject to rigid controls.

Concomitant changes in living conditions

Because of the possibility that other uncontrolled variables in Bataan have affected the results of the experiment, we have carefully examined changes in economic conditions. On the whole some improvement was in progress during the experimental period. Sales tax collections increased 50% in area A and 138% in area B between two comparable 9-month periods, beginning July 1, 1947, and July 1, 1949. Total provincial revenue increased 39% from 1947–1948 to 1949–1950 (estimated). There was an estimated increase of 107,200 in the number of tins of milk (14 oz.) consumed (1.3 gm per capita daily). There were several small-scale distributions by relief agencies of food products, milk, spaghetti, rice, beans and fish, but the total amount was trivial (about 1 lb. per

capita during two entire years) and it was distributed approximately equally during the pre-experimental and experimental periods and in both areas.

It is difficult to believe that the results can have been affected materially by these extraneous factors.

Plans for the future

A premix plant has been erected in Manila by the United States Public Health Service for the National Rice and Corn Corporation, and the rice mills of Tarlac and other beriberi-endemic provinces are being progressively equipped with mixing machinery for producing enriched rice. It will not be possible to conduct extensive clinical surveys of these larger populations, but continued collection of mortality statistics in these provinces as well as in Bataan should furnish further evidence concerning the public health value of enrichment.

Correlated information on blood, urine and food analysis is being collected for future publication. Analyses of blood samples taken in Bataan at the end of the pre-experimental period appear in a recent publication (Burch et al., '50).

SUMMARY

The substantially exclusive use of enriched rice by 63,000 people in Bataan since October 1, 1948, indicates, by clinical examinations of about 12,000 persons prior to and subsequent to inauguration of enrichment, a decline of 76 to 94% in the incidence of beriberi in each of 7 municipalities (weighted average, 89%). By contrast, in an adjacent control area (29,000 population) the incidence rose somewhat during the same period.

Beriberi mortality has similarly declined from 263 per 100,000 in the experimental area in 1947–1948 to 28 per 100,000 in 1949–1950, the latter rate being only a small fraction of the lowest annual rate ever previously recorded for Bataan.

Infantile beriberi mortality declined 53% in the experimental area during the year during which the introduction of enriched rice took place, although enrichment was in effect only during the last 9 months of that year.

There is evidence, of a less conclusive character, of the favorable effect of enriched rice on mortality from all causes and specifically on mortality from tuberculosis.

It is hoped to extend rice enrichment to 1,800,000 additional people by the end of 1951.

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VITAMIN A UTILIZATION STUDIES

IV. RELATIVE UTILIZATION OF VITAMIN A ALCOHOL, VITAMIN A ACETATE AND VITAMIN A NATURAL ESTERS AS AFFECTED BY VARIATIONS IN DOSAGE LEVEL, QUANTITY AND CHARACTER OF DILUENT, AND QUANTITY OF DIETARY FAT

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In previous vitamin A utilization studies performed with rats, the present authors investigated the factors affecting hydrolysis *in vivo* of vitamin A esters, particularly in respect to their varying effects on the relative utilization of differing chemical forms of the vitamin as determined by liver storage measurements. These experiments were performed at high levels of vitamin A intake on rats that had been reared on a high-fat basal diet. It was shown that, in addition to other factors, the relative biological efficacy of the alcohol and ester forms of vitamin A was greatly affected by the quantity and nature of the diluent present in the vitamin A supplements administered.

It was the purpose of the present study to determine the effect of mineral oil and ethyl laurate, used as diluents, on the utilization of various forms of vitamin A by the rat. The effects of the fat content of the basal ration and the level of vitamin A dosage on relative utilization of differing forms of vitamin A were also investigated.

EXPERIMENTAL

The vitamin A alcohol, acetate and natural ester concentrates were prepared using male soupfin shark liver oil as

the source of vitamin A. The natural ester concentrate was made by a process which does not involve the use of high vacuum distillation. The spectrophotometric and chemical characteristics of the concentrates used in the following experiments fell within the limits for such preparations previously described.

The dilutions of vitamin A in corn oil,[1] mineral oil (U.S.P.) and ethyl laurate[2] were made on the basis of vitamin A spectrophotometric units ($E^{1\%}_{1\,cm}$, 328 mμ × 2,000) and contained 5% mixed tocopherols.

Sprague-Dawley male rats, 21 days of age, were used in all experiments. The animals were maintained on either a high-fat or a low-fat basal ration for the duration of the test. The high-fat diet consisted of 18% casein, 45% cornstarch, 8% brewers' yeast, 4% U.S.P. salt mixture 2, 25% hydrogenated vegetable oil,[3] 0.125% vitamin E (mixed tocopherols), 60 units of vitamin D and 1.5 units of vitamin A per 10 gm of feed. The groups of rats on the low-fat diet received the U.S.P. XIII Vitamin A Assay Basal Diet (5% fat content) with the addition of 1.5 units of vitamin A per 10 gm of feed. The rats were placed on test when 50 to 60 days of age.

The liver of each rat was assayed for vitamin A, using a modification of the procedure developed by Glover et al. ('47). Vitamin A values were determined by the Carr-Price reaction, using the Evelyn colorimeter standardized with the U.S.P. Vitamin A Reference Oil. These procedures are fully described elsewhere (Week and Sevigne, '49a).

Experiment 1

Rats, 54 days of age, reared on the high-fat basal ration were divided into comparable groups of 8 each according to weight. The rats were supplemented orally, by means of a calibrated tuberculin syringe, with 0.4 ml of the respective vitamin A supplement daily for three successive days. Each

[1] Mazola.
[2] Eastman grade.
[3] Primex.

rat received a total of 9,000 units of vitamin A for the three-day period. The rats were rested on the 4th day and killed on the following day. The livers were stored under refrigeration until assayed for vitamin A. Control analyses indicated no loss of vitamin A during the period of storage.

Experiment 2

When 54 days of age, rats maintained on the high-fat ration were divided into comparable groups by weight and fed 0.4-ml daily supplements of the respective vitamin A preparations, 6 days a week, for a period of 5 weeks. Each rat received a total of 3,000 units of vitamin A for the 5-week period. At the conclusion of the supplementing period the rats were killed and the livers removed and stored under refrigeration until assayed for vitamin A, as in experiment 1.

Experiment 3

Two groups of rats were maintained on the 25% and the 5% fat diets, respectively. When 56 days of age both groups were divided into comparable subgroups of 10 rats each and supplemented with the various vitamin A preparations, as shown in table 5. Each rat received a total of 9,000 units of vitamin A for the three-day period. The rats were supplemented daily for three days, rested for 24 hours, and killed on the 5th day. The livers were removed and assayed for vitamin A as described in previous experiments.

DISCUSSION OF RESULTS

Experiment 1

The analytical results are listed in table 1. These data were subjected to analysis of variance in order to determine the significance of the values obtained (table 2).

For the groups listed in table 1, it is possible to make 36 separate comparisons of two groups taken at a time. Nineteen of these comparisons disclose statistically significant

TABLE 1

Relationship between chemical forms of vitamin A and diluent oils, as measured by liver storage by the rat [1]

Vol. and potency of supplement fed daily	CORN OIL 0.4 ml @ 7,500 units/ml			MINERAL OIL 0.4 ml @ 7,500 units/ml			ETHYL LAURATE 0.4 ml @ 7,500 units/ml		
Total units fed in 3-day period	9,000			9,000			9,000		
Group no.	1	2	3	4	5	6	7	8	9
Form of vitamin A	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester
Rat no.									
1	2,575	1,900	2,815	2,450	2,165	2,675	1,700	2,620	2,100
2	2,200	2,440	2,975	2,540	2,715	1,995	1,825	1,825	1,490
3	2,400	2,925	2,575	2,700	2,815	1,545	1,980	1,850	1,770
4	2,725	2,840	2,600	2,425	1,850	2,515	1,890	1,980	2,530
5	2,925	3,125	2,040	3,050	1,730	1,900	1,500	1,945	2,070
6	2,975	3,165	2,275	3,050	2,415	2,620	1,475	1,730	2,040
7	3,150	2,790	2,815	2,390	2,425	2,390	1,465	1,730	2,055
8	2,115	3,225	2,175	3,050	2,795	2,415	2,225	2,245	2,260
Average vitamin A units per liver	2,633	2,801	2,534	2,707	2,364	2,257	1,758	1,990	2,039
Average vitamin A stored (%)	29.3	31.1	28.2	30.1	26.3	25.1	19.5	22.1	22.7
Coefficients of variation (%)	14.3	15.8	13.3	11.1	17.7	17.7	15.6	15.3	15.2

[1] A difference of 353 units is required for significance (5% level) between any two test groups. A difference of 468 units is required for significance (1% level) between any two test groups. Control groups, receiving diluent oils only as a supplement, showed no liver storage of vitamin A.

differences, of which the following are considered to be of interest: group 1 with 7; 2 with 5 and 8; 3 with 9; 4 with 6 and 7; and 5 with 8. Certain of the comparisons which do not disclose significant differences are also of interest: group 1 with 2, 3 and 4; 2 with 3; 3 with 6; 4 with 5; 5 with 6; 6 with 9; 7 with 8 and 9; and 8 with 9.

In our previous vitamin A utilization studies with chicks (Week and Sevigne, '49a) ethyl laurate was shown to have a significant effect on the utilization of all three vitamin A forms relative to their utilization from corn oil. As is shown

TABLE 2

Analysis of variance for data listed in table 1

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARES	REMARKS
Diluent	2	3,349,833	Highly significant $P < 1\%$
Individuals	7	73,488	
Form of vitamin A	2	80,430	
Diluent × individuals	14	122,492	
Diluent × form of vitamin A	4	344,807	Significant $P < 5\%$
Individuals × form of vitamin A	14	107,490	
Residual	28	151,503	
Pooled error	65	125,186	

in table 1, this effect is also evident when rats are used; however, with rats this inferior utilization can be attributed to factors adversely affecting stability *in vivo* or absorption per se, or both, in addition to a probable inhibition of vitamin A ester hydrolysis by ethyl laurate.

Comparison of group 1 with group 4 shows that mineral oil does not have an effect on vitamin A alcohol significantly different from that of corn oil, thereby demonstrating that, for the rat, the difference between the combined effects of the stability and absorption per se factors in mineral oil versus those of the same factors i corn oil is not significant.

The comparisons of group 2 with 5 and group 3 with 6 demonstrate that mineral oil inhibits the hydrolysis of vitamin A esters to a greater extent than does corn oil, although, in the case of the natural ester form (group 3 with 6), the difference is not significant at the 5% level.

In the comparison of group 1 with 3 and group 4 with 6, the utilization of vitamin A alcohol is compared to that of the natural ester in the same carriers. The results show that when mineral oil serves as the carrier, the significantly lower utilization of the natural ester form can only be due to factors involving hydrolysis. When corn oil was used as the diluent, no significant difference in utilization between the two chemical forms was observed.

In general, these results also confirm our previous conclusion (Week and Sevigne, '49a, b) that factors influencing the hydrolysis *in vivo* of vitamin A esters may be of equal or greater significance than stability factors, so far as their effect on the relative biological response of differing forms of vitamin A is concerned.

Experiment 2

The analytical data and the analysis of variance are shown in tables 3 and 4, respectively.

For the experimental groups listed in table 3, it is possible to make 36 separate comparisons of two groups taken at a time. Twenty-two of these comparisons disclose differences which are statistically significant (5% level), of which the following are considered to be of interest: group 1 with 3 and 7; 2 with 5 and 8; 3 with 6 and 9; 4 with 7; 5 with 8; 7 with 9; and 8 with 9. Of the comparisons which do not disclose significant differences, the following are of interest: group 1 with 2; 2 with 3; 4 with 5 and 6; 5 with 6; 6 with 9; and 7 with 8.

As in experiment 1, the comparison of group 1 with group 4 shows that the difference between the combined effects of stability and absorption per se factors in mineral oil versus those of the same factors in corn oil was not significant.

TABLE 3

Relationship between chemical forms of vitamin A and diluent oils, as measured by liver storage by the rat [1]

Vol. and potency of supplement fed daily	CORN OIL 0.4 ml @ 250 units/ml			MINERAL OIL 0.4 ml @ 250 units/ml			ETHYL LAURATE 0.4 ml @ 250 units/ml		
Total units fed in 5 weeks, 6 days a week	3,000			3,000			3,000		
Group no.	1	2	3	4	5	6	7	8	9
Form of vitamin A	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester
Rat no.									
1	344	473	680	353	295	405	150	235	335
2	419	460	655	505	525	368	200	325	415
3	468	543	523	568	368	473	195	200	453
4	655	453	678	450	388	398	180	263	440
5	553	830	398	353	418	478	135	158	285
6	298	523	508	478	425	490	333	223	408
7	540	418	733	433	455	365	215	220	225
8	560	548	535	515	373	395	288	255	415
Average vitamin A units per liver	480	531	589	457	406	422	212	235	372
Average vitamin A stored (%)	16.0	17.7	19.6	15.2	13.5	14.1	7.1	7.8	12.4
Coefficients of variation (%)	25.1	24.4	19.4	16.7	16.7	12.1	31.7	20.9	22.0

[1] A difference of 89 units is required for significance (5% level) between any two test groups. A difference of 118 units is required for significance (1% level) between any two test groups. Control groups, receiving diluent oils only as a supplement, showed no liver storage of vitamin A.

The very signifiant difference between groups 3 and 6 is therefore shown to be due to factors affecting hydrolysis *in vivo*. Thus, our conclusions as to the significance of hydrolytic factors in influencing the relative biological responses of differing forms of vitamin A at high levels of dosage prove to be valid at dosage levels only one-thirtieth those of our original experiments.

However, the comparisons of group 1 with 3, group 4 with 6 and group 7 with 9, as contrasted with comparisons between the same group numbers in experiment 1, show that the dosage

TABLE 4

Analysis of variance for data listed in table 3

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARES	REMARKS	
Diluent	2	411,137	Highly significant	$P < 1\%$
Individuals	7	5,023		
Form of vitamin A	2	44,218	Highly significant	$P < 1\%$
Diluent × individuals	14	7,529		
Diluent × form of vitamin A	4	22,495	Significant	$P < 5\%$
Individuals × form of vitamin A	14	6,438		
Residual	28	9,491		
Pooled error	63	7,880		

level is a significant factor in the relative utilization of differing forms of vitamin A.

The longer term of experiment 2 as compared to experiment 1 was adopted simply to allow for sufficient liver stores to accumulate so that assay errors would not be unreasonably large relative to absolute values. It is clear that the length of the experiment cannot affect the relative utilization of vitamin A alcohol, acetate and natural esters; the change is due only to the change in level of dose.

The change in relative utilization of the different vitamin A forms was in the direction of favoring the natural ester at the lower dosage level. The relative utilization of vitamin

A alcohol and acetate was remarkably uniform at the two different dosage levels under the conditions of this experiment.

On the basis of the relative improvement of vitamin A natural esters versus vitamin A alcohol at lower dosage levels, one might be tempted to conclude that factors affecting hydrolysis *in vivo* assume diminished importance at low dosage levels, or factors affecting stability *in vivo* assume increased importance, or both. However, the authors believe that factors affecting both hydrolysis and stability *in vivo* may exercise proportionately greater influence on vitamin A utilization at low dosage levels. In a sense, both of these groups of factors operate with greater intensity when the vitamin A dose is small. Their relative influence usually seems to be affected by a change in dosage level and it is probable that the change in relative influence with change in dose is in the direction of giving greater weight to the factors affecting stability.

It was found in experiment 2 that the ethyl laurate produced severe irritation of the membranes of the mouth and throat of the rats when administered over the 5-week period. This irritation became noticeable after the first week of the supplementing period, when lesions in the oral cavity developed. Examination of the internal organs after the rats were killed showed no abnormalities, and therefore the liver storage measurements for these experimental groups are included. It should be noted, however, that the rats receiving vitamin A supplements containing ethyl laurate showed a 15% smaller growth response for the 5-week period than rats fed corn oil or mineral oil.

Experiment 3

The analytical data and the analysis of variance are shown in tables 5 and 6.

For the liver storage measurements shown in table 5, it is possible to make 66 separate comparisons of two groups taken at a time. Twenty-seven of these comparisons disclose statistically significant differences (5% level), of which the following are believed to be important: group 1 with 2; 2 with

TABLE 5

Relationship among chemical forms of vitamin A, fat content of diet and volume of corn oil in the supplement fed, as measured by liver storage by the rat [1]

DIET	25% FAT						5% FAT					
Vol. and potency of supplement fed daily	0.1 ml @ 30,000 A/ml			0.4 ml @ 7,500 A/ml			0.1 ml @ 30,000 A/ml			0.4 ml @ 7,500 A/ml		
Total units fed in 3-day period	9,000			9,000			9,000			9,000		
Group no.	1	2	3	4	5	6	7	8	9	10	11	12
Form of vitamin A	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester	Alcohol	Acetate	Ester
Rat no.												
1	3,140	3,000	3,300	2,275	3,025	2,765	3,050	4,040	3,555	3,215	3,140	3,010
2	3,180	3,650	3,325	3,450	2,715	3,015	3,090	3,125	3,190	3,075	4,215	3,150
3	3,150	3,490	3,400	2,840	3,215	2,675	3,715	3,865	3,375	3,350	3,075	3,125
4	3,020	3,525	3,175	3,490	3,500	3,240	3,100	3,450	3,265	2,900	3,000	3,275
5	3,240	3,540	3,030	2,875	2,800	3,015	2,840	3,415	3,040	3,050	2,975	3,000
6	3,290	3,450	3,525	3,475	3,315	3,050	3,450	3,300	3,040	2,915	3,150	3,040
7	3,090	3,190	3,475	2,725	2,940	2,915	3,375	3,525	3,200	3,315	2,840	2,840
8	3,450	4,000	3,590	3,450	2,765	2,565	3,025	3,640	3,075	3,150	2,765	2,900
9	3,000	2,925	3,165	2,825	3,540	2,890	3,250	3,540	3,350	2,850	2,840	2,425
10	2,840	3,575	3,315	2,765	3,425	2,490	3,320	4,015	3,765	3,140	3,500	3,265
Average vitamin A units per liver	3,140	3,435	3,330	3,017	3,124	2,862	3,222	3,592	3,286	3,096	3,150	3,003
Average vitamin A stored (%)	34.9	38.2	37.0	33.5	34.7	31.8	35.8	39.9	36.5	34.4	35.0	33.4
Coefficients of variation (%)	5.38	9.11	5.21	13.96	10.09	8.23	7.62	8.21	6.91	5.54	13.62	8.25

[1] A difference of 319 units (1% level) or 241 units (5% level) is required for significance between any two test groups. Control groups, receiving diluent oils only as a supplement, showed no liver storage of vitamin A.

5; 3 with 6; 5 with 6; 7 with 8; 8 with 9 and 11; and 9 with 12.

Of the possible comparisons that do not show a significant difference, the following are of interest: group 1 with 3, 4 and 7; 2 with 3 and 6; 3 with 9; 4 with 5, 6 and 10; 5 with 11; 6 with 12; 7 with 9 and 10; 10 with 11 and 12; and 11 with 12.

TABLE 6

Analysis of variance for data listed in table 5

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARES	REMARKS
Volume of diluent	1	2,555,001	Highly significant $P < 1\%$
Individuals	9	95,433	
Form of vitamin A	2	563,778	Highly significant $P < 1\%$
Diet	1	161,333	
Vol. diluent × individuals	9	99,881	
Vol. diluent × form of vitamin A	2	210,633	
Vol. diluent × diet	1	2,253	
Diet × individuals	9	175,439	Significant $P < 5\%$
Diet × form of vitamin A	2	5,036	
Individuals × form of vitamin A	18	45,531	
Vol. diluent × individuals × diet	9	118,037	
Vol. diluent × form of vitamin A × diet	2	63,345	
Vol. diluent × form of vitamin A × individuals	18	51,054	
Individuals × form of vitamin A × diet	18	46,060	
Residual	18	101,950	
Pooled error	107	74,247	

From these group comparisons and the analysis of variance shown in table 6, the following conclusions can be made from the data in table 5: The comparison of group 1 with 4, when contrasted with the comparisons of group 2 with 5 and group 3 with 6, confirms the findings of previous experiments (Week and Sevigne, '49b) that the change from 0.1 ml to 0.4 ml of corn oil diluent has a significantly adverse effect on the hydrolysis of the vitamin A esters. The comparison

of group 7 with 10, when contrasted with the comparisons of group 8 with 11 and group 9 with 12, shows that the adverse effect of larger quantities of corn oil diluent on vitamin A ester hydrolysis is significant when the diet of the test animals contains 5% fat as well as when it contains 25% fat.

The comparisons of groups 1 with 7, 2 with 8, 3 with 9, 4 with 10, 5 with 11 and 6 with 12 show that no significant difference in utilization of any of the forms of vitamin A is produced by changing the fat content of the diet from 25% to 5%. In 5 of these 6 comparisons, however, vitamin A was somewhat better utilized with the low-fat diet.

SUMMARY

1. The effects were investigated of variations in the type and quantity of diluent, the fat content of the basal diet and the level of vitamin A intake on the relative biological utilization of vitamin A alcohol, acetate and natural esters by the rat. Liver storage measurements were used as the criterion of utilization.

2. Mineral oil and ethyl laurate were shown to contain factors which inhibit vitamin A ester hydrolysis *in vivo*.

3. The vitamin A dosage level is shown to be a significant factor in the relative utilization of differing forms of vitamin A by the rat. The change in the relative biological efficacy appears to favor the natural ester form at the lower dosage level.

4. It was demonstrated that the biological utilization of vitamin A alcohol is not significantly affected by (1) varying from 0.1 ml to 0.4 ml the quantity of corn oil diluent contained in the vitamin A supplement; (2) varying from 5% to 25% the fat content of the basal ration; or (3) an interaction between these two factors.

5. Increasing the amount of corn oil diluent decreased significantly the utilization of vitamin A esters, irrespective of the fat content of the diet.

6. Increasing the fat content of the diet did not significantly affect the utilization of vitamin A acetate and vitamin A natural esters, irrespective of the quantity of diluent.

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THE HEMOGLOBIN CONCENTRATIONS, ERYTHROCYTE COUNTS, AND HEMATOCRITS OF SELECTED LOUISIANA ELEMENTARY SCHOOL CHILDREN

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THREE FIGURES

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For almost three decades the close alliance of nutrition and hematology has been recognized. Because of the interdependence of these two sciences, hematologic technics have been used not only in studies of deficiency states, but also in assessing nutritional condition.

Major problems for those interested in the nutritional status of a population are the relative importance of various nutritional factors, and the establishment of indices that could be used effectively in assessing the nutriture of people. If hematological values are indices of nutritional status, it is the responsibility of research workers in this field to determine the level of these blood values of the segment of the population they are studying. In order to interpret the significance of these hematological levels, research must also reveal the relative importance of these blood values to the nutriture of people, and identify those values closely associated with growth and the improvement and maintenance of health.

Many of the nutritional status studies reported to date were studies of college students, families of industrial workers, and children living in orphanages. Comparatively few

data have been reported on children, living in their own family groups, in the pre-adolescent and early adolescent stages. The body demands for growth at these stages are great, and information concerning the nutriture of children in these age groups is needed.

In the present study, the investigators were interested in ascertaining whether or not there were significant differences in the hematological status of Louisiana children within the different agricultural areas of the state. A wide variation in dietary patterns from one agricultural area to another was noticeable. An effort was made to make some contribution of basic data needed in ascertaining nutritional condition, to provide more information on the average blood values of children in pre-adolescence and early adolescence, and to ascertain to some degree the hematological status of these Louisiana children.

This investigation of the hematological values of Louisiana school children was a part of a larger nutritional status study conducted at Louisiana State University during the school years of 1947–48 and 1948–49. It was limited to a study of 515 white elementary school children, aged 8 to 13 years. The hematological values determined were hemoglobin concentrations, erythrocyte counts, and hematocrit values. From these determinations calculations were made for the mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

PROCEDURE

The sampling for the study was done on the basis of the distribution within the state of Louisiana elementary school children. The state was divided into 4 large agricultural areas according to the types of soil and principal crops: area A, South Louisiana sugar and truck farming; area B, South Louisiana rice and general farming; area C, North Louisiana hills; and area D, North Louisiana delta. The number of children selected from each agricultural area was in the

proportion of the elementary school population of the area to that of the entire state. The bases for the selection of the schools in each area were the accessibility of the school to the University laboratory, the willingness on the part of the school to cooperate in the project, and the cooperation of parish health units in furnishing trained nurses for the drawing of samples of the venous blood. The children within the specified age groups from 14 schools were used in the study.

For laboratory analyses, a 10-ml sample of blood was drawn from an antecubital vein of each subject between 9:00 and 10:30 A.M. Before the venipuncture a tourniquet was applied about two inches above the bend of the elbow of the subjects and efforts were made to prevent stasis, with care being taken not to apply the tourniquet too tightly nor to leave it on longer than two minutes. The blood was expelled from the syringe into a glass collecting tube which contained a dried anticoagulant. The anticoagulant was made by preparing a solution containing 0.8 gm of dried potassium oxalate and 1.2 gm of dried ammonium oxalate in 100 ml of distilled water, with the addition of 1 ml of 40% formalin to prevent deterioration. This proportion of ammonium oxalate and potassium oxalate has been shown to prevent shrinkage in the volume of red cells and changes in other corpuscular constituents (Wintrobe, '46). Unless the samples were collected in the Nutrition Research Laboratory of Louisiana State University, the tightly stoppered tubes of blood were packed around cans of ice in an insulated bag and transported immediately to the Laboratory. If the blood samples were not collected in the Laboratory, the distance from the school to the Laboratory determined the length of time between the collection of the blood and the performance of the tests. Whenever possible the tests were made within three hours from the time the blood was collected. In some cases it was not possible to make the tests until the following morning. In these cases all tests were made within 24 hours. At the time of analysis of each sample the previously refrigerated blood was brought to room temperature. To assure complete mix-

ing before the blood sample was withdrawn from the collecting tubes, the tubes were well, but gently, shaken by end-over-end inversion.

The hemoglobin determinations were done in duplicate by the acid hematin method (Wintrobe, '46) and read in the Fisher electro-hemometer. For erythrocyte counts, certified Thoma diluting pipettes and a Spencer haemacytometer with improved Neubauer double ruling were used (Wintrobe, '46). Two complete dilutions with two counts, agreeing within 20,000 cells, were averaged for the final count. For the packed cell volume, Wintrobe hematocrit tubes were filled with blood by use of a capillary pipette. Then the tubes were centrifuged for 30 minutes at 2,500 revolutions per minute. The volume of packed red cells was read from the scale on the tube, and an average reading of duplicate samples was taken as the final value.

Calculations were made for the mean corpuscular volume, the mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration according to the formulae given by Wintrobe ('46).

In the statistical analyses of the data, the standard error of the difference between the means was computed for all combinations of ages and areas using the following formula:

$$\sigma\, \mathrm{d} = \sqrt{\sigma^2 \bar{x}_1 + \sigma^2 \bar{x}_2}$$

The use of this formula constituted a very rigorous test in that it was based on the assumption that there was no correlation between one age and another; if such a relationship existed, the standard error of the difference between the means would have been even smaller, resulting in a larger critical ratio.

FINDINGS AND RESULTS

Hemoglobin values

Using Macy's standard ('42) of 13.0 ± 1 gm per 100 ml for hemoglobin values, 86.9% of the Louisiana subjects were

normal, with values above 12.0 gm per 100 ml, and 13.1% of the subjects were below normal. Of these, 3.5% were below 11.1 gm per 100 ml, and 9.6% were between 11.1 and 12.0 gm per 100 ml. Variations were found in the standards accepted as normal hemoglobin values. Wintrobe ('46) gave normal values of 12.9 $\pm$ 2.0 gm per 100 ml for children 6 to 10 years of age, and 13.4 $\pm$ 2.0 for children 11 to 15 years of age. The normal value accepted by Metcoff and co-workers ('45) in a Newfoundland study was 12.0 gm per 100 ml. In study-

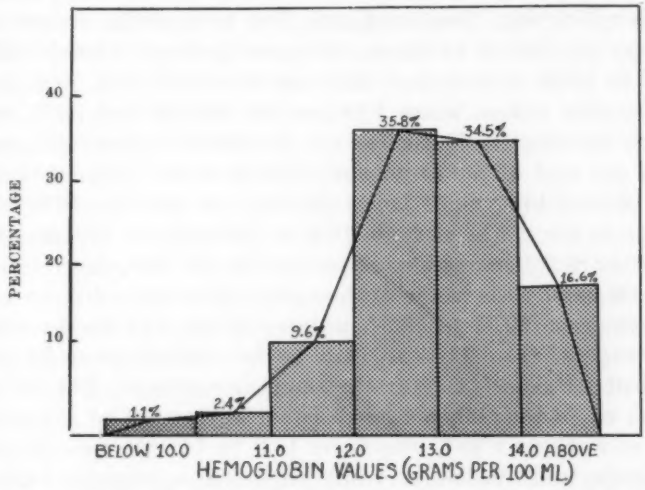

Fig. 1 Percentage distribution of subjects at various hemoglobin levels.

ing children 6 to 18 years old in Florida, Abbott, Townsend and Ahmann ('45) used the following criteria: 13.6 gm per 100 ml, normal; 11.4 to 13.6 gm per 100 ml, subnormal; 3.6 to 11.4 gm per 100 ml, anemia.

The frequency distribution of the hemoglobin values of the subjects at various hemoglobin levels is presented in figure 1. The largest percentage of the subjects, 35.8%, had values of from 12.1 to 13.0 gm per 100 ml, while the values of 34.5% were from 13.1 to 14.0 gm per 100 ml, and of 16.6%

were above 14.0 gm per 100 ml. Smaller percentages were seen at the lower levels: 9.6% of the values were from 11.1 to 12.0 gm per 100 ml, 2.4% were from 10.1 to 11.0 gm per 100 ml and 1.1% were below 10.0 gm per 100 ml. The curve shown in figure 1 was skewed negatively, with the majority of the values falling above the 12.0 gm per 100 ml level.

In a study of hemoglobin values in Pennsylvania, Mack et al. ('41) reported that 82.0% of the boys and 80.2% of the girls had values above 11.5 gm per 100 ml. Metcoff and associates ('45) reported that 81% of the children in their study of Norris Point, Newfoundland, had hemoglobin values of 12.0 gm per 100 ml or above. From a study of Florida children in 1939, Abbott and Ahmann reported that 50% had hemoglobin values below 9.64 gm per 100 ml and 30% had values of from 11.74 to 9.64 gm per 100 ml. One and one-tenth per cent of the Louisiana children in the present study had hemoglobin values below 10.0 gm per 100 ml; 12% had values in the range of from 10.0 to 12.0 gm per 100 ml; and the other 86.9% had values above 12.0 gm per 100 ml.

In this study the range of the mean values according to age groups, from 12.77 to 13.34 gm per 100 ml, was higher than the range of from 11.47 to 12.90 gm per 100 ml given by Osgood and Baker ('35) for the same age groups. The range of the mean for children aged 9 to 13, reported by Kaucher and co-workers ('48), was from 13.0 to 14.0 gm per 100 ml. Mugrage and Andresen ('36) reported a slightly higher range, of from 13.84 to 14.53 gm per 100 ml. The mean hemoglobin values for Louisiana boys in the present study was 13.1 gm per 100 ml, as compared to 12.65 gm per 100 ml for the boys studied by Milam and Muench ('46). The Louisiana girls had a mean hemoglobin value of 13.1 gm per 100 ml, while the girls studied by Milam and Muench had a mean of 12.54 gm per 100 ml.

The Louisiana children showed a larger percentage of values in the higher hemoglobin levels than the Florida and the Pennsylvania children. The mean hemoglobin values of the Louisiana children were slightly higher than those re-

ported by Milam and Muench ('46) and Osgood and Baker ('35). The range of the means of the children in this study was slightly narrower than that reported by Kaucher and co-workers ('48) and by Mugrage and Andresen ('36).

On the basis of the differences between the mean values as compared to the standard error, the differences between age and sex groups were not statistically significant. The 9-year-old boys had the lowest mean hemoglobin value, 12.77 ± 1.04 gm per 100 ml, while the 12-year-old boys had the highest, 13.34 gm ± 0.98 gm per 100 ml (table 1). The greatest difference between boys and girls of a single age group was in the 9-year-old group, where the girls had a mean hemoglobin value of 13.16 gm per 100 ml, and the boys a value of 12.77 gm per 100 ml. The mean hemoglobin value for the entire group of subjects was the same as the means for both the boys and the girls; namely, 13.1 gm per 100 ml.

The results are in agreement with those reported by other investigators. Mugrage and Andresen ('36) reported that there were some variations between the hematological values for boys and girls in this age group, but they were not considered significant. Osgood and Baker ('35) reported no significant variations with respect to sex or age with subjects 8 to 13 years of age. Abbott et al. ('45) reported that differences in the values for girls and boys were slight before the age of 12.

In comparing the hemoglobin values of the agricultural areas, there was only 0.4 gm difference between the highest mean hemoglobin value, 13.4 gm per 100 ml, found in area B, and the lowest, 13.0 gm per 100 ml, found in area D. Table 2 presents the mean and range of the hemoglobin values for each area. Although areas A and C had the same mean value, 13.1 gm per 100 ml, area A had a wider range, of from 6.4 to 15.8 gm per 100 ml, as compared with the range of area C of from 9.1 to 16.0 gm per 100 ml.

Brown and Goodall ('46) found that individuals showed variations from day to day averaging 0.93 gm, with a maximum variation of as much as 2 gm. Kaucher and associates

TABLE 1

Percentage distribution, by age and sex, of 99% of the subjects at various hemoglobin levels

HEMOGLOBIN	NUMBER OF CASES	PERCENTAGE OF CASES	PERCENTAGE OF SUBJECTS AT VARIOUS HEMOGLOBIN LEVELS									
			Age 9		Age 10		Age 11		Age 12		Age 13	
			Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
gm/100 ml												
Under 10.0	6	1.1	..	..	1.5	1.5	2.4	2.4	..	..	..	..
10.1 to 11.0	13	2.4	4.5	..	1.5	3.0	2.4	3.5	1.4	1.4	3.2	4.5
11.1 to 12.0	52	9.6	18.8	5.0	10.5	11.6	10.7	8.3	6.8	8.5	9.6	9.0
12.1 to 13.0	194	35.8	33.3	50.0	38.9	40.6	31.0	36.4	38.4	30.0	38.7	22.7
13.1 to 14.0	187	34.5	33.4	30.0	29.9	31.8	39.2	33.0	30.1	40.0	35.5	45.5
Over 14.0	90	16.6	9.5	15.0	17.9	11.6	14.3	16.5	23.3	20.0	12.9	18.2
Total number of cases	542 [1]	100.0	21	20	67	69	84	85	73	70	31	22
Mean hemoglobin	13.1		12.77	13.16	13.10	12.83	13.09	12.98	13.34	13.28	13.08	13.23
Standard deviation	± 1.14		1.04	0.87	1.08	1.17	0.99	1.04	0.98	0.90	0.93	1.56

[1] The number of cases includes both a spring and fall examination of some of the subjects.

TABLE 2

Hemoglobin concentrations, erythrocyte counts, hematocrit values, and MCV, MCH, MCHC [1] values of subjects according to agricultural areas

AREAS	NUMBER OF SUBJECTS	MEAN	S. D.	RANGE
		Hemoglobin values		
A	180	13.1	1.17	6.4–15.8
B	88	13.4	0.98	11.1–15.8
C	135	13.1	1.25	9.1–16.0
D	112	13.0	0.93	11.0–15.0
All subjects	515	13.1	1.14	6.4–16.0
		Erythrocyte counts		
A		4.77	0.46	3.24–5.80
B		5.00	0.41	4.45–5.86
C		4.79	0.47	3.79–5.79
D		4.94	0.28	4.20–5.97
All subjects		4.89	0.58	3.79–5.86
		Hematocrit values		
A		41.4	1.77	37.1–47.0
B		41.3	3.34	35.0–58.0
C		42.7	2.86	31.0–48.8
D		40.5	3.52	32.0–52.0
All subjects		41.3	4.52	31.0–58.0

AREAS	MCV		MCH		MCHC	
	Mean	Range	Mean	Range	Mean	Range
			Calculated values			
A	89	76–109	27	17–34	31	26–37
B	84	68–116	26	21–31	31	25–37
C	89	63–117	26	21–33	31	21–41
D	81	65–108	26	23–29	32	21–44
All subjects	85	63–117 [2]	27	19–33	31	21–44 [3]

[1] MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

[2] Less than 1% of the cases were above 109 μ^3.

[3] Less than 1% of the cases were above 38%.

('48) found an average variation of 0.7 gm with children who were observed on consecutive days. Because of the variability introduced by the method of estimation, as well as true physiological changes, Mole ('45) stated that unless a change of 8% is found in serial determinations of the hemoglobin level, such a change cannot be considered significant. None of the differences found between boys and girls, age groups, and agricultural areas in this study was as large as 8%.

TABLE 3

Percentage distribution of 97% of the subjects at various erythrocyte count levels

ERYTHROCYTE COUNT	NUMBER OF CASES	PERCENTAGE OF CASES	PERCENTAGE OF SUBJECTS AT VARIOUS ERYTHROCYTE COUNT LEVELS AT AGE				
			9	10	11	12	13
million red cells/mm³ blood							
5.51–6.00	36	7.0	7.9	4.6	5.0	7.9	16.0
5.01–5.50	162	31.3	28.9	30.0	30.6	35.0	28.0
4.51–5.00	235	45.4	47.5	46.2	46.3	44.3	42.0
4.01–4.50	79	15.2	15.7	16.1	16.8	12.8	14.0
3.51–4.00	6	1.1		3.1	1.3		
Total number of cases	518	100.0	38	130	160	140	50
Mean	4.89		4.90	4.84	4.87	4.95	4.99
Standard deviation	0.58		0.47	0.47	0.36	0.39	0.48

Erythrocyte counts

Of the total group of subjects, 45.4% were found to have erythrocyte counts of from 4.51 to 5.00 millions per mm³, while 31.3% had values between 5.01 and 5.50 million per mm³ and 15.2% had values between 4.01 and 4.50. These percentages are shown in table 3 with the distribution of the subjects at various erythrocyte levels.

Macy ('42) established a normal erythrocyte count of 4.76 ± 0.41 million per mm³ for children of this age, while Wintrobe ('46) arrived at a normal count of 4.7 ± 0.6 million per mm³ for children aged 6 to 10 years, and of 4.8 ± 0.6 mil-

lion per mm³ for 11- to 15-year-old children. According to these standards, 8.1% of the subjects in the present study had values outside of the normal range. Those were the 1.1% in the interval of from 3.51 to 4.00 million per mm³, and 7.0% in the interval of from 5.51 to 6.00 (fig. 2).

The variations among the erythrocyte counts for the age groups were not statistically significant. The highest mean of the erythrocyte counts was found in the 13-year-old group,

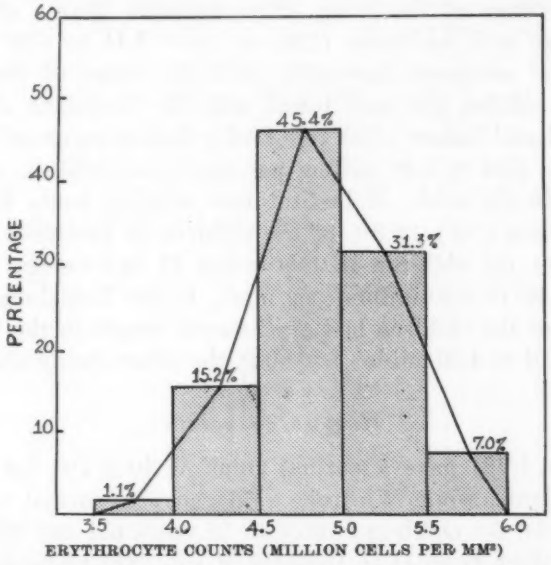

Fig. 2 Percentage distribution of subjects at various erythrocyte levels.

with a mean of 4.99 ± 0.48 million per mm³. The lowest was 4.84 ± 0.47 million per mm³, with the 10-year-old group.

The mean and range of the erythrocyte counts for each area are presented in table 3. Area B had the highest mean, 5.00 million per mm³, with a range of from 4.45 to 5.86; area D was second with a mean of 4.94 million per mm³ and a range of from 4.20 to 5.97. Area C was third with a mean of 4.79 million per mm³ and a range of from 3.79 to 5.79, and

area A had the lowest mean, 4.77 million per mm^3, with a range of from 3.24 to 5.80. The only significant difference among the mean erythrocyte counts for the 4 agricultural areas was between area A and area B. The ranges indicated that the variations among individual subjects were great and that the erythrocyte counts of some of the subjects were below the accepted normal, yet all of the mean values of the groups were within the normal range.

The range of the mean of erythrocyte counts given by Mugrage and Andresen ('36) of from 4.44 to 4.58 million per mm^3 compares favorably with the range of from 4.84 to 4.99 million per mm^3 found with the Louisiana children. Osgood and Baker ('35) reported a higher range of means, of from 5.04 to 5.22 million per mm^3, for children of these ages. In the study of institutional children made by Mack and others ('47), 96.8% of the children in Institution I and 100% of the children in Institution II had values ranging from 4.00 to 4.50 million per mm^3. In the Louisiana group, 15.2% of the children had erythrocyte counts in the interval from 4.01 to 4.50 million per mm^3, the others being above this interval.

Hematocrit values

Macy ('42) gave a normal value of $40 \pm 7\%$ for packed cell volume, while Wintrobe ('46) gave a normal value of 37.5 ± 5.0 for children from 6 to 10 years old and 39.0 ± 5.0 for children from 11 to 15 years of age. The percentage distribution of the hematocrit values according to age is shown in table 4 and figure 3. Both the 11- and 12-year-old groups had a mean of 41.2%, while the 10- and 13-year-old groups each had a mean of 41.6% and the mean for the 9-year-old group was 41.3%. None of the differences among the mean values of the age groups was significant. The mean hematocrit values for boys and girls indicated that, as was true for the hemoglobin determinations and erythrocyte counts, the differences between the sexes were not consistent.

TABLE 4

Percentage distribution of 95% of the subjects at various hematocrit levels

HEMATOCRIT VALUES	NUMBER OF CASES	PERCENTAGE OF CASES	PERCENTAGE OF SUBJECTS AT VARIOUS HEMATOCRIT VALUE LEVELS AT AGE				
			9	10	11	12	13
%/100 ml							
47.1–51.0	14	3.7	7.4	6.8	1.6	1.0	7.3
43.1–47.0	89	23.6	22.2	17.1	25.8	26.8	24.4
39.1–43.0	195	51.8	40.7	59.1	50.9	50.5	48.8
35.1–39.0	68	18.0	29.7	15.9	17.7	18.6	14.6
31.1–35.0	11	2.9	...	1.1	4.0	3.1	4.9
Total number of cases	377 [1]	100.0	27	88	124	97	41
Mean hematocrits	41.3		41.3	41.6	41.2	41.2	41.6
Standard deviation	± 4.52		4.88	3.22	3.74	3.64	4.81

[1] Hematocrits were obtained on 400 subjects.

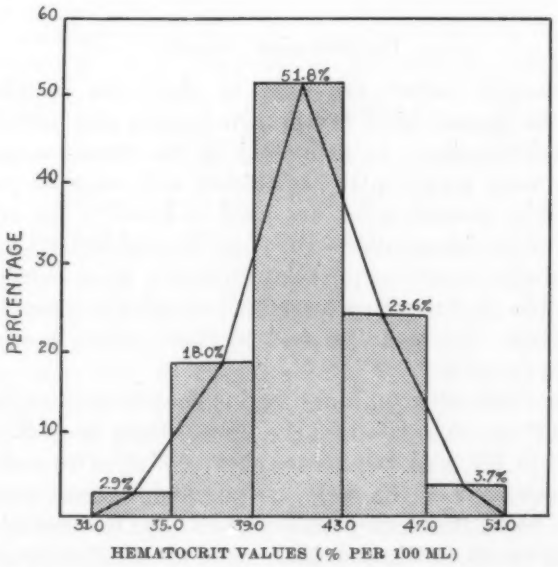

Fig. 3 Percentage distribution of subjects at various hematocrit levels.

The agricultural areas ranked in the following order according to mean hematocrit values: Area C, 42.7%; area A, 41.4%; area B, 41.3%; area D, 40.5%. The differences between the mean values for areas A and D, and areas C and D, were large enough to be significant.

Osgood and Baker ('35) gave the range of the mean values for these ages as being 35.82 to 38.05%. Mugrage and Andresen ('36) gave it as being from 40.90 to 43.15%. With the Louisiana children the range of the mean values of these age groups, from 41.2 to 41.6%, was smaller than that of the other two studies. Mack et al. ('47) reported 44.7% of the Pennsylvania children in Institution I, and 63.4% of the children in Institution II, had hematocrit values in the interval from 37 to 39%. These workers also stated that the hematological values of the Pennsylvania children indicated that the majority of them, in both orphanages, originally had a mild or severe hypochromic anemia.

Corpuscular values

Corpuscular values are used to show the relationships among the hemoglobins, erythrocyte counts, and hematocrits. These relationships, as expressed by the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, are used to identify the types of anemia. The abbreviations MCV, MCH, and MCHC are used to designate mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, respectively. The mean for each of these values in each area is shown in table 2.

Of the total subjects, 14.6% had mean corpuscular volumes ranging from 60 to 80 μ^3, 55.1% ranged from 81 to 92, 29.3% from 93 to 109, and 1.0% were above 109 μ^3. One and three-tenths per cent of the mean corpuscular hemoglobins were from 15 to 21 micro micrograms, 42.8% were from 21 to 26, 51.8% from 27 to 31, and 4.1% were above 31 micro micrograms. There were 2.8% of the cases which had mean cor-

puscular hemoglobin concentrations below 25%, 29.5% were from 25 to 30%, 65.5% were from 31 to 36%, and 1.2% were from 36 to 38%. There were 4 cases, or about 1% of the subjects, who had mean corpuscular hemoglobin concentrations above 38%.

According to Sturgis ('48), microcytic hypochromic anemia may usually be identified by a combination of low mean corpuscular values; specifically, a MCV within the range of from 60 to 80 μ^3, a MCH within the range of from 15 to 21 micro micrograms, and a MCHC range of from 25 to 30%. There

TABLE 5

Comparison of mean hematological values of Louisiana children with accepted standards

BLOOD VALUES	WINTROBE'S STANDARDS ('46)	MACY'S STANDARDS ('42)	LOUISIANA CHILDREN	
			Mean	S. D.
Hemoglobin gm per 100 ml	12.9 ± 2	13.0 ± 1	13.1	± 1.14
Erythrocyte counts million per mm^3	4.7 ± 0.6	4.76 ± 0.4	4.89	± 0.58
Hematocrits % per 100 ml	37 ± 5	40.0 ± 7	41.3	± 4.52
MCV μ^3	80 ± 5		85	
MCH $\mu\mu g$	27 ± 2		27	
MCHC %	34 ± 2		31	

were 14.6% of the present subjects who had a MCV of from 60 to 80 μ^3; 1.3% who had a MCH of from 15 to 21 micro micrograms; and 29.5% who had a MCHC of from 25 to 30%.

Wintrobe ('46) gave a MCV greater than 94 μ^3 and a MCHC greater than 30% as characteristics of a macrocytic condition, while a MCV of less than 80 μ^3 and a MCHC of greater than 30% were given as characteristic of a simple microcytic condition. A MCV of less than 80 μ^3 and a MCHC of less than 30% were stated to be characteristic of a hypochromic microcytic condition. Of the total group of Louisiana children, 3.7% fell into the first classification. Seventeen and nine-tenths per cent of the values fell into the second classification,

and 2.4% of the Louisiana children had values described in the third classification. There are other clinical symptoms that would have to be considered in the identification of anemia. No clinical examinations were made of these subjects to determine such symptoms, and no attempt was made to describe these hematological conditions as being indicative of various types of anemia. Table 5 presents a summary of the mean hematological values for the Louisiana children and compares them with those reported by Wintrobe and Macy.

SUMMARY AND CONCLUSIONS

For 515 Louisiana school children, aged 8 to 13, the mean hemoglobin value was 13.1 $\pm$ 1.14 gm per 100 ml, with a range of from 6.4 to 16.0. The mean erythrocyte count was 4.89 $\pm$ 0.58 million per mm^3, with a range of from 3.79 to 5.86. For the hematocrits the mean was 41.3 $\pm$ 4.52%, with a range of from 31 to 58. For the MCV the mean was 85 μ^3, with a range of from 63 to 117. The mean of the MCH was 27 micro micrograms, with a range of from 19 to 31. For the MCHC, the mean was 31% and the range was from 21 to 44%. One per cent of the cases accounted for mean corpuscular volumes above 109 μ^3 and mean corpuscular hemoglobin concentrations above 38%.

The mean hematological values were within the generally accepted normal range for children of this age group, and indicated a wide variation among individuals. The differences between the age groups and sexes were not statistically significant. With the hemoglobin values, there were no significant differences among the mean values for the areas. With the erythrocyte counts, there was a significant difference between areas A and B. The differences between the mean hematocrit values of areas A and D, and areas C and D, were also significant.

Of the total group, 86.9% of the hemoglobin values, 91.9% of the erythrocyte counts, and 96.3% of the hematocrit values were within the generally accepted normal range for children

from 8 to 13 years of age. The corpuscular values were compared to the ranges of these values given by Sturgis ('48) and also by Wintrobe ('46) in classifying anemias. Seventy-five per cent of the Louisiana children were normal according to these standards.

The hematological values of this group of Louisiana children were higher than those reported for children studied in Florida, Pennsylvania, and Oregon. They compared favorably with the hematological values reported for children in North Carolina, Michigan, and Colorado.

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VITAMIN A STORES OF SUCKLINGS FOLLOWING ADMINISTRATION TO THE DAMS OF VITAMIN A IN OILY AND AQUEOUS MEDIA [1]

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It was reported earlier from this laboratory that aqueous dispersions of vitamin A are better absorbed than oily solutions (Sobel et al., '46). This finding has been amply confirmed since then by: (1) the changes in serum vitamin A reported by some workers (Kramer et al., '47; Sobel et al., '49); (2) liver storage studies (Sobel et al., '48b); (3) balance studies (Lewis et al., '47, '50); and (4) vitamin A fluorescence studies of the intestinal tract (Popper and Volk, '48). More recently it has been shown that aqueous dispersions of vitamin A administered orally to lactating women and to milk cows caused a far greater rise in milk vitamin A concentration than the same amount of vitamin A dissolved in oil (Sobel, Rosenberg and Kramer, '48a, '50; Sobel et al., '49). The question arose as to whether this increase in milk vitamin A concentration represents increased vitamin A for the nursing young.

To resolve this problem experimentally, the vitamin A stores of suckling rats whose dams received vitamin A in aqueous dispersion were compared to the A stores of sucklings whose dams received the same vitamin A in oily solution.

[1] Supported in part by a grant from Endo Products, Inc., New York, N. Y.

EXPERIMENTAL

Albino rats of the inbred Wistar strain fed the stock diet of Bills et al. ('31) were used. The pregnant dams employed were litter-mate pairs. After parturition, one of the litter-mate dams received a daily test dose of vitamin A in aqueous dispersion and the other received the same test dose in oily solution. (In one case more oily solution than aqueous dispersion was given.)

The daily dose was normally given for 4, 5, or 6 days through a stomach tube, employing the technique of Shay and Gruenstein ('46). The young were sacrificed 24 hours after the final dose. The sucklings were frozen solid with dry ice in alcohol (three to 4 seconds immersion sufficed) and the livers and kidneys excised while still frozen solid. This technique allows the removal of the intact organs with no contamination from body fluids or adhesion of adjacent tissues.

Control studies have shown that of the total vitamin A in the carcass at least 80% is contained in the liver and kidneys, some of the remaining 20% being in the gastrointestinal tract. It may be worthwhile pointing out, however, that in a newborn rat the liver and kidneys contain only from 50 to 65% of the total vitamin A stores.

The whole liver and kidneys were placed in a test tube and treated with 1.0 to 2.0 ml of approximately one normal potassium hydroxide in 95% aldehyde-free ethyl alcohol and digested at 60°C. until a homogenous solution was obtained. This required approximately 30 minutes. To this hydrolysis mixture an equal volume of distilled water was added. The vitamin A was then extracted and evaluated with glycerol dichlorhydrin by the process described for milk by Sobel and Rosenberg ('49).

The preparation employed was the unsaponifiable fraction of fish liver oil containing 800,000 to 1,000,000 U.S.P. units of vitamin A per gram. This material was diluted to contain the daily dose in 0.20 ml: (a) in maize oil; and (b) in water containing 16% of the dispersing agent described by Sobel

et al. ('48b).[2] Thus the only variable between the two vitamin A preparations employed was the medium.

Vitamin A transfer to young as measured by combined liver and kidney stores

In the first series of experiments the mothers continued to take the stock diet of Bills et al. ('31), which is rich in

TABLE 1

Comparison of vitamin A stores in the livers and kidneys of sucklings whose dams[1] were fed vitamin A in aqueous and oily media

GROUP	MEDIUM	A/DAY	NO. DAYS	NO. ANIMALS/ LITTER	A/LITTER	A/ SUCKLING (Mean)
		U.S.P. units			*μg*	*μg*
A	Aq. disp.	2,000	4	9	28.7	3.2
	Maize oil	2,000	4	8	16.2	2.0
B	Aq. disp.	2,000	5	5	33.2	6.6
	Maize oil	2,000	5	5	22.7	4.5
C	Aq. disp.	1,500	4	5	12.9	2.6
	Maize oil	2,000	4	6	7.3	1.2
D	Aq. disp.	2,000	6	9	41.6	4.6
	Maize oil	2,000	6	6	26.8	4.5
E	Aq. disp.	2,000	4	6	16.3	2.7
	Maize oil	2,000	4	6	1.4	0.23
F	Aq. disp.	2,000	6	5	42.3	8.5
	Maize oil	2,000	6	5	29.2	5.8
Mean	Aq. disp.	1,917	4.8	6.5	29.2	4.7
	Maize oil	2,000	4.8	6.0	17.3	3.1

[1] Dams maintained on diet of Bills et al. ('31).

vitamin A, in order to simulate the conditions obtaining in well-fed mothers. The results of this study are shown in table 1. The group receiving aqueous A had higher stores for each litter-mate pair in every case, the mean storage per litter in the "aqueous" group being 29.2 μg of vitamin A as compared to 17.3 μg in the "oily" group. In the control litters analyzed,

[2] The product is called "ACon."

the A storage was from 4 to 6 μg per litter. The low stores found when the dam received no supplement suggested that if such a study were undertaken on dams fed a vitamin A-deficient diet, the difference would be greater.

Depletion of the dams during the gestation period, while desirable, is not practical because abnormalities in the young may present themselves (Warkany, '44). We decided, therefore, to place the post partum mothers on a vitamin A-free diet. The U.S.P. XIII Vitamin A Test Diet was employed.

TABLE 2

Comparison of vitamin A stores in the livers and kidneys of sucklings whose dams[1] *received vitamin A in aqueous and oily media*

GROUP	MEDIUM	A/DAY	NO. DAYS	NO. ANIMALS/ LITTER	A/LITTER	A/ SUCKLING
		U.S.P. units			μg	μg
G	Aq. disp.	2,000	4	9	52.2	5.8
	Maize oil	2,000		12	14.4	1.2
H	Aq. disp.	2,000	4	10	41.7	4.2
	Maize oil	2,000		11	13.3	1.2
I	Aq. disp.	2,000	4	4	39.3	9.8
	Maize oil	2,000		7	11.2	1.6
J	Aq. disp.	2,000	4	6	28.5	4.8
	Maize oil	2,000		6	2.2	0.4
Mean	Aq. disp.	2,000	4	7.3	40.4	6.4
	Maize oil	2.000	4	9.0	10.3	1.1

[1] Dams maintained on U.S.P. XIII vitamin A test diet.

Dams who were litter mates were given 2,000 units of vitamin A per day in an aqueous dispersion or oily solution. The results of this study are given in table 2. The group receiving aqueous A again had higher stores for each littermate pair in every case, the mean stores per litter in the "aqueous" group being 40.4 μg as compared to 10.3 μg in the "oily" group. Thus the availability of vitamin A to the suckling young was approximately 4 times as great when the dams were fed vitamin A in an aqueous dispersion as when the same amount of A was given in an oily solution.

DISCUSSION

The present experiments show that aqueous dispersions of vitamin A are far more effective in enabling the suckling young to store this vitamin than a corresponding amount of A given in oil. The explanation must lie in the improved absorption of vitamin A in an aqueous dispersion and the far more efficient enrichment of milk with aqueous dispersions, as has been shown for both cows and humans (Sobel, Rosenberg and Kramer, '50). It has been possible to relate this enrichment to the higher blood levels that one obtains with aqueous dispersions. The total amount of vitamin A found in the livers and kidneys of the sucklings was small compared to that which the mothers received. Whether this represents limited vitamin A secretion by the dam, poor absorption by the sucklings of the amount present in the milk, or the inability of the young organism to store more than a limited amount of vitamin A is a subject for further investigation. Studies of liver storage with respect to age have shown that such storage is definitely limited (Guerrant, '49). Calculations made on the basis of our work with humans and cows show a transfer to the milk when aqueous dispersions are used of approximately 1 to 3% of the administered vitamin A. In the cow this increased transfer, as measured by milk levels, takes 48 hours; in humans, apparently, the transfer is completed in 24 hours. In the present studies we can assume a milk transfer of about 40 μg of vitamin A per day, beginning on the second day. This indicates a total transfer of approximately 140 μg. Of this, the sucklings stored approximately 40 μg, which represent about one-third of the vitamin A received since the last dose probably did not find its way into the milk. One can thus estimate that 30% is stored from the milk, which represents the same order of magnitude as the vitamin A storage of older rats given aqueous dispersions of vitamin A (Sobel et al., '48b). While these calculations are tentative, the results indicate that there is not only secretion of more vitamin A into milk following

administration of aqueous dispersions, but that, of this increased amount, far more is available to the suckling young.

The true significance of these findings for the human species remains for further work to determine.

SUMMARY

The vitamin A stores of suckling rats whose dams were given vitamin A in an aqueous dispersion were about 4 times as great as the stores of suckling rats whose dams received the same amount of vitamin A dissolved in oil. In view of previous studies on milk transfer, the conclusion may be drawn that orally ingested vitamin A in an aqueous dispersion is more effectively transferred to milk and stored in the suckling than vitamin A in an oily solution.

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SUPPLEMENTATION OF WHEAT WITH AMINO ACIDS IN THE DIET OF THE CHICK[1]

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The supplementary nutritive value of amino acids for various proteins, and the specific effects of amino acid deficiencies on growth rate, nitrogen utilization, and excretion, have been studied extensively. Particularly with animals other than chicks, considerable research has been carried out on the relationship between amino acid intake and nitrogen balance. In this regard Anderson and Nasset ('48) found that reducing the quantity of either isoleucine or methionine in the rat diet led to decreased efficiency of nitrogen utilization. Similarly, Sauberlich, Pearce and Baumann ('48) found that when mice were fed a diet in which the protein was incomplete, they were not able to use nearly as much of the protein eaten as they did when it was complete. It was noted that when an incomplete protein was fed, increased quantities of amino acids appeared in the urine.

Since wheat contributes a major portion of the protein content in many poultry rations, it seemed of importance to determine whether the protein requirement of growing chicks could be met largely by wheat of high protein content upon appropriate amino acid supplementation, thus obviating the need for protein concentrates in the ration. This, of course, would be possible only if adequate quantities of vitamins and minerals were present in the ration. In the following experi-

[1] This project was carried out under a grant from the British Columbia Research Council, Vancouver, Canada; Dr. S. E. Maddigan, Director.

ments, the effects of amino acid supplementation of wheat samples of different protein content were studied. Particular attention has been given to the efficiency of nitrogen utilization as evidenced by over-all feed efficiency and by the excretion of amino acids and total nitrogen. It has been demonstrated repeatedly that since lysine is the primary limiting amino acid in wheat protein, supplementation of wheat with lysine increases the nutritive value of this grain for various animals. For this reason emphasis has been placed on the effects of lysine additions to an all-wheat chick diet.

EXPERIMENTAL

The first test in the series was of an exploratory nature to determine the extent to which an all-wheat chick ration may be improved by the addition of lysine. In the second test, the growth response of chicks to different levels of lysine added to an all-wheat ration was measured with respect to both growth and feed efficiency. In both tests I and II, the wheats were supplemented with vitamins and minerals so that 100 lb. of the final ration contained (in pounds): butyl fermentation solubles 1.5, limestone 2.0, iodized salt 1.0, feeding oil (2,400 A, 400 D) 0.5, cereal grass 2.0, choline chloride (25% preparation) 0.44, manganese sulphate 6 gm, riboflavin 0.1 gm, and calcium pantothenate 0.2 gm. The basal rations were supplemented as indicated in table 1. The various diets were fed ad libitum to day-old White Leghorn chicks reared in lots of 13 to 18 in electric battery brooders. A record was kept of weekly weight gains and feed consumption.

Test III was designed to ascertain the effects of excesses of lysine as well as of two other amino acids, methionine and tryptophan, which were known to be present in the ration at levels beyond the requirement at the limiting level of lysine. In this test, day-old New Hampshire cockerel chicks were fed a well-balanced commercial chick starter ration for 10 days. The chicks were weighed on the 5th and the 10th days, and those showing extreme deviation from the average weight were discarded. Eight lots of 10 chicks each were made up

TABLE 1

The effect of lysine supplementation [1] of wheat in the chick diet

	PROTEIN CONTENT OF WHEAT [2]	LYSINE SUPPLEMENT TO DIET	AVE. WT. OF CHICKS AT 4 WKS OF AGE	EFFICIENCY OF FEED UTILIZATION
	%	gm/lb.	gm	Feed / Gain
Test I	11.9	..	82	5.9
	11.9	1.8	110	3.7
Test Ia	11.9	..	83	7.4
	11.9	1.8	127	5.0
	16.5	..	95	6.0
	16.5	0.9	127	4.7
Test II	11.3	..	92	4.3
	11.3	0.5	120	..
	11.3	1.0	138	2.1
	11.3	1.5	140	2.1
	11.3	2.0	145	2.2

[1] Added to the rations as L(+) lysine monohydrochloride.

[2] The protein values given were calculated using the N × 5.7 factor.

and distributed in electrically heated battery brooders with wire screen floors. They were fed the following ration, supplemented as indicated in table 2: ground wheat 93.6 lb., butyl fermentation solubles 2.0 lb., limestone 1.0 lb., bonemeal 2.0 lb., iodized salt 0.5 lb., feeding oil (1,200 A, 400 D) 0.5 lb., choline chloride (25% preparation) 0.44 lb., manganese sulphate 6 gm, riboflavin 0.05 gm, calcium pantothenate 0.10 gm, and niacin 0.15 gm.

From the 10th to the 14th day a record was kept daily of weight gains, feed consumption, and feces excretion. In order to obtain consistent weights, it was found necessary to weigh the birds in the morning before they had access to feed and water. After having been on the experimental ration for 4 days, the chicks appeared to have become accustomed to the new rations and were gaining weight uniformly within each lot. The data given below were for the ensuing 10 days.

During this 10-day period the chicks were weighed on alternate days. The feces were collected daily, frozen imme-

TABLE 2

Total nitrogen consumption and excretion of chicks fed amino acid supplements (test III)

RATION NO. Supplement/lb. of basal ration	1 ..	2 1 gm L(+) lysine HCl	3 2 gm L(+) lysine HCl	4 3 gm L(+) lysine HCl	5 1 gm DL-methio-nine	6 2 gm DL-methio-nine	7 0.5 gm DL-trypto-phan	8 1 gm DL-trypto-phan
Ave. wt. (gm)	111	147	143	131	107	108	115	112
$\frac{\text{Feed}}{\text{gain}}$	4.26	3.19	3.46	3.64	6.18	5.31	4.91	4.83
$\frac{\text{Feed N} - \text{Fecal N}}{\text{gain}}$	0.032	0.030	0.033	0.035	0.047	0.040	0.037	0.038
Fecal N (%)	4.58	4.01	4.21	4.38	4.72	4.72	4.73	4.76
$\frac{\text{Fecal N}}{\text{Feed N}}$ (%)	59	51	52	53	60	60	60	59

diately, and dried, before analysis, under vacuum at 65 to 70°C. The nitrogen content of the pooled feces for each two-day period was determined by the Kjeldahl method. The free ammonia remaining in the feces after drying was determined and the percentage of nitrogen present in the feces corrected for it. Thus the nitrogen excretion figures given in table 2 represent the total nitrogen excreted minus the amount present as ammonia under similar conditions of collection and treatment.

The feces collected during the last two days of the test were analyzed for their content of lysine, methionine, and tryptophan according to the method of Henderson and Snell ('48). The total amounts of these amino acids were determined on acid hydrolysates of the feces in the case of lysine and methionine, and on alkaline hydrolysates in the case of tryptophan. The results given in table 3 are the average results of analyses on duplicate hydrolysates. The quantities of lysine, methionine, and tryptophan which were excreted in the free form were determined on unhydrolyzed water extracts of the feces. Extraction was accomplished by shaking

TABLE 3

Lysine, methionine and tryptophan consumption and excretion of chicks (test III)

SUPPLEMENT PER LB. RATION	% OF FECAL N FROM			AMINO ACID EXCRETED / AMINO ACID CONSUMED %			FREE AMINO ACID / TOTAL AMINO ACID % IN FECES		
	Lysine	Methionine	Tryptophan	Lysine	Methionine	Tryptophan	Lysine	Methionine	Tryptophan
....	1.03	2.01	2.85	22.0	16.4	18.3	6.7	20.8	9.3
1.0 gm L(+)lysine HCl	0.86	2.06	2.77	9.8	14.7	15.6	5.1	16.8	8.6
2.0 gm L(+)lysine HCl	1.23	2.23	3.20	10.4	16.6	18.6	4.0	13.4	7.5
3.0 gm L(+)lysine HCl	1.25	2.04	2.57	8.7	16.1	15.9	10.0	22.3	8.7
1.0 gm DL-methionine	0.98	1.89	2.26	21.4	9.0	14.9	8.5	27.9	11.7
2.0 gm DL-methionine	1.22	2.05	2.68	27.3	6.9	17.8	5.6	26.5	12.6
0.5 gm DL-tryptophan	0.99	1.98	3.19	21.9	16.6	14.7	6.3	16.2	18.7
1.0 gm DL-tryptophan	1.01	2.13	3.46	21.8	17.5	11.9	7.6	17.6	24.2

5.0 gm of the dried samples with 50 ml water in the presence of toluene for 12 hours.

In test IV (table 4) wheats of different protein content were used to study further the efficiency of wheat protein utilization when the wheats were supplemented with herring meal to yield rations containing 17 to 21% of total protein. Mineral and vitamin supplements were added to meet the

TABLE 4

Average weights of chicks fed wheat of different protein content supplemented with herring meal

	PROTEIN CONTENT OF WHEAT [1]	PROTEIN CONTENT OF RATION	AVE. WT. OF CHICKS AT 4 WKS. OF AGE	EFFICIENCY OF FEED UTILIZATION
	%	%	gm	$\frac{Feed}{Gain}$
Test IV	11.9	unsupplemented	83	7.4
	11.9	18	224	2.4
	16.5	unsupplemented	95	6.0
	16.5	18	145	2.9
Test IVa	11.3	17	224	2.8
	13.2	17	215	2.5
	16.5	17	109	4.1
	11.3	19	236	2.3
	13.2	19	220	2.3
	16.5	19	189	2.9
	11.3	21	252	2.1
	13.2	21	250	2.4
	16.5	21	229	2.5

[1] The protein values given for the wheat were calculated using the N × 5.7 factor.

National Research Council allowances. Day-old White Leghorn cockerel chicks, 13 per lot, in test IV, and day-old New Hampshire cockerel chicks, 16 per lot, in test IVa, were used.

RESULTS AND DISCUSSION

The results of tests I and II (table 1) showed that an essentially all-wheat ration could be improved by the addition of lysine. The chicks grew decidedly faster on the wheat ration which had been supplemented with lysine, and the

efficiency of feed utilization was improved up to 20%. The growth rate was improved with increasing levels of lysine up to 1.0 gm per pound of diet with the particular sample of wheat included in this ration. Above this level neither growth rate nor feed efficiency could be improved by the further addition of lysine.

In test III (table 2), as in test II, 1.0 gm lysine per pound of ration appeared to be the optimum amount required to make up the deficiency in the wheat, since the addition of lysine in excess of this quantity tended to lower the growth response. On the other hand, addition of methionine or tryptophan, which were already present in the diet in excess, did not increase the growth rate of the chicks.

With respect to feed utilization, there was a marked rise in efficiency with the addition of the limiting amino acid, i.e., lysine. When, however, the amount of lysine added was in excess of the quantity required for maximum growth with the diet concerned, the efficiency began to fall off. A similar situation was encountered when either methionine or tryptophan, which were already present in the ration in excess (at the low level of lysine present), was added. Thus, although the addition of either of these amino acids to the ration had no effect on growth, each of them lowered the efficiency of the already poorly utilized diet. The fact that the excess lysine did not affect the efficiency of feed or nitrogen utilization to the same extent as methionine or tryptophan may possibly be explained by the fact that lysine is poorly re-absorbed by the kidneys (Silber, Seeler and Howe, '46), and thus an excess does not interfere to the same extent as do methionine or tryptophan with the re-absorption of the other amino acids.

Interpretation of the data on amino acid excretion is complicated by the fact that, with poultry, fecal and urinary nitrogen are excreted together. The term "fecal" nitrogen, as used herein, includes both fecal and urinary nitrogen. When the amino acid balance of the ration was improved by the addition of lysine, the nitrogen content of the feces, as well as the percentage of feed nitrogen excreted in the feces,

decreased. When, however, lysine was added to the ration in excess, the fecal nitrogen content increased. Methionine or tryptophan supplementation of the lysine-deficient wheat diets resulted in an increase in the fecal nitrogen content, since the amino acid imbalance in the ration was aggravated by the addition of amino acids already in excess relative to the amount of lysine present. It will be seen in table 2 that, in addition to causing an increase in the fecal nitrogen content, an imbalance of amino acids in the diet also resulted in a decrease in the efficiency of the nitrogen metabolized (feed N minus fecal N) as regards gain in weight.

With respect to the excretion of the individual amino acids, there was a marked decrease in the percentage of the lysine, methionine and tryptophan intake which was excreted when these amino acids were added to the ration (table 3). This decrease occurred despite the fact that there were no variations in the percentages of the fecal nitrogen which could be attributed to the addition of these amino acids to the diet. The lack of correlation between the amounts of lysine, methionine and tryptophan in the diet and the amounts present in the feces is in agreement with the reports of Shen and Lewis ('46) and Eckert and Allison ('47) that, with dogs, cats, rabbits and rats, any excess methionine present in the diet is metabolized. The constant amino acid content of the feces, together with the decrease in the percentage of the lysine, methionine and tryptophan intake to be excreted (increase in per cent metabolized), indicates that when amino acids are added to the chick diet they are metabolized at the expense of the other amino acids in the ration.

The data presented above on the supplementation of a lysine-deficient diet with lysine, methionine, and tryptophan, respectively, indicate that, from the aspect of nitrogen utilization, a deficency of a single amino acid corresponds, in effect, to an excess of the other amino acids at the level of the limiting one. Hence, adding tryptophan or methionine to the all-wheat ration under consideration increased the excess of amino acids present in relation to the lysine content of

the ration, and lowered the efficiency of nitrogen utilization when the ration was fed to the chick. The depressing effect of the added methionine and tryptophan on the efficiency of nitrogen utilization was not considered to be the result of using the DL- forms of these amino acids. In this connection, it has recently been reported by Van Pilsum and Berg ('50) that with the rat an excess of methionine in the L- form may be more deleterious than such an excess in the D- form. They suggested that the smaller growth retardation observed with the feeding of the D- form may result from its more ready escape into the urine.

The data obtained in the present experiment on the effects of excess amino acids in the presence of a deficiency of another amino acid are comparable in some respects to the results obtained by Grau ('48) on the effect of protein level on the lysine requirement of the chick. Grau reported that as the protein level of a chick diet was increased, the lysine requirement of the chick for maximum growth was also raised. With a suboptimum amount of lysine at any given protein level, growth was found to be greater than with the same amount of lysine at a higher protein level. Apparently at the higher protein level the amino acids which were in excess in the diet (relative to the level of lysine) were instrumental in producing a lower rate of growth.

With respect to the practical aspect of the problem of supplementing wheat rations, it will be seen from table 4 that the protein content of the wheat used affected the requirement for supplementary protein. In the case of a high protein wheat, in this instance 16.5%, the amount of fish meal required to bring the total ration to 17 to 21% protein was not sufficient to compensate for the amino acid deficiency of the wheat. The difference between the 11.3% and the 13.2% protein levels was less marked, there being no difference when sufficient fish meal was added to bring the protein content of the rations up to 21%. There were, however, slight differences in favor of the 11.3% wheat at the 17 and 19% levels. These results are similar to those obtained by McElroy and

Draper ('49) working with pigs fed rations containing grains of different protein content. These workers observed that when a protein supplement was not included in a swine ration, the protein content of the grain affected the growth rate and feed efficiency; and, further, that when a protein supplement was fed, high protein grain resulted in a certain saving in the amount of protein supplement required.

It would appear on the basis of the present and other studies (Biely and March, '49) that wheat of a high protein content, unless properly supplemented (with appropriate amino acids), has little growth-promoting value over wheat of average protein content (11 to 13.0%).

In formulating rations containing large proportions of wheat, caution must be exercised in protein supplementation, particularly where the wheat used is high in protein. In the latter case, it may even be necessary to have a ration higher in total protein than would be the case if a lower protein wheat were used. Poor growth and feed efficiency on a ration containing high protein wheat may result from a combination of two factors: (1) an actual amino acid deficiency; and (2) excesses of certain amino acids interfering with the efficient utilization of those amino acids present in lesser, although not necessarily limiting, amounts.

SUMMARY

Different samples of wheat have been used as the sole source of protein in chick rations. The effects of supplementation of the all-wheat diets with lysine, methionine, tryptophan, and fish meal have been studied with regard to growth rate and feed utilization. It was found that:

1. The addition of lysine to an all-wheat ration increased the growth rate of chicks and improved the efficiency of feed utilization.

2. Lysine in excess of the optimum level for the particular amino acid composition of the ration decreased both the growth rate and the efficiency of feed utilization.

3. The addition of methionine or tryptophan to an all-wheat ration depressed the efficiency of feed utilization.

4. Although the efficiency of nitrogen utilization was markedly decreased by the presence of excesses of amino acids in the diet, nevertheless the ratio of fecal to dietary nitrogen did not change at the amino acid levels present in the experimental rations.

5. When wheat samples of different protein content were fed in rations containing no additional protein concentrates, growth rate and feed efficiency were slightly better with wheat of high protein content. High protein wheat (16.5%), however, gave slower growth and lower feed efficiency than wheat of average protein content (11 to 13%) when supplemented with fish meal to yield rations containing 17 to 21% of total protein.

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THE NITROGEN-SPARING EFFECT OF DIETARY CARBOHYDRATE IN ITS RELATION TO THE TIME FACTOR. EXPERIMENTS WITH REPLETION OF PROTEIN-DEPLETED ADULT RATS[1]

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ONE FIGURE

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Larson and Chaikoff ('37), working with adult dogs, and Cuthbertson et al. ('40) and Munro ('49), studying adult humans and rats, showed that the N-sparing effect of a single carbohydrate feeding was dependent upon the time which elapsed between the feeding of the protein and carbohydrate moieties of the diet. These authors found that if sugar was fed more than 4 hours before or after the protein meal, no N-sparing effect could be demonstrated.

The above workers do not offer any explanation for the N-sparing effect. Larson and Chaikoff emphasize, however, that "the nitrogen spared is not permanent, its elimination beginning several hours after its storage is effected." Further, it was found that when carbohydrate and protein were fed simultaneously, the improvement of the N balance was only transitory; the effect can be demonstrated only in the first few days, after which time the N balance returns to its original level.

[1] Supported by grants from the Research Corporation, Williams-Waterman Fund, and the National Vitamin Foundation.

It was conceivable that the N-sparing effect of simultaneously fed sugar was only transitory because in adult, well-fed animals the capacity to store protein is only transitory, and after the stores have been filled, the nitrogen fed in excess of the daily requirement is excreted. In growing animals, where the amino acids of the dietary protein can be utilized for the formation of new tissue, the simultaneous feeding of sugar and protein should have a prolonged effect. Basing his work on such considerations, one of the present authors (Geiger, '48) performed experiments on infantile rats and found that the simultaneous feeding of these food constituents actually improved growth for the duration of the 21-day experiment.

In order to investigate this point further, the effect of the time factor on the repletion of protein-depleted adult rats has been studied.

METHODS

Adult male rats of the Sprague-Dawley strain were used. Each animal was placed in an individual, round metabolism cage. Urine and feces were collected before the morning feeding. Urine was stored with the addition of a few drops of sulphuric acid and toluene. The N content of the excreta pooled for a period of 4 days was determined by the method of Kjeldahl. In feeding the animals and in the composition of diets I to IV, the method of Munro ('49) was generally followed. The composition of the diets is given in table 1.

The rats were depleted over a period of 18 days. In order to enhance the mobilization of body protein, the animals were starved every second day during this period and on the alternate days had free access to the practically protein-free "depletion diet."

At the end of the depletion period the animals were divided into two groups. Group A received diet I in the morning and diet II in the evening; i.e., the glucose and protein moieties were fed separately. Group B, on the other hand, received diet III in the morning and diet IV in the afternoon. In this group carbohydrate and protein were fed simultaneously.

The morning diets (I and III) were offered at 9:00 A.M. and the afternoon diets (II and IV) at 4:00 P.M. Both diets were eagerly consumed and the cups with final small residues were removed two hours after they were made accessible to the animals.

The total N content of the daily diet was 337 mg; i.e., during the 4-day period of each N balance experiment a total of 1,350 mg N was consumed.

TABLE 1

Composition of diets used

COMPONENT	DIET							
	I	II	III	IV	V	VI	VII	VIII
	gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Butter	2.8	..	2.8	..	..	..	4	4
Glucose	4	..	..	4	..	4	4	..
Casein [1]	..	2.5	..	2.5	3.0	3.0	..	..
$NaHCO_3$	..	0.15	..	0.15	0.15	0.15	..	..
Basal diet [2]	2	...	2	...	...	...	2	2

[1] The casein was made into a paste with water and $NaHCO_3$, following Munro's suggestion.

[2] With the following composition: cornstarch 51.6%, Woodflock (finely powdered wood of low resin content which has been extracted with water and solvents) 14.4%, cod liver oil (U.S.P., Nutritional Biochem. Corp., Cleveland) 14.6%, wheat germ oil (Viobin Corp.) 4%, U.S.P. salt mixture II 15.4%, Ca-pantothenate 5 mg %, inositol 25 mg%, choline chloride 250 mg %, niacin 2.5 mg %, thiamine 1.2 mg %, riboflavin 1.5 mg %, pyridoxine 1.2 mg %, biotin 0.2 mg %, folic acid 0.3 mg %.

The "depletion diet" had a composition as follows: cornstarch 3,050 gm (78.3%), rice bran concentrate 400 gm (10.26%), cottonseed oil 200 gm (5.13%), U.S.P. salt mixture 200 gm (5.13%), fish oil (1 gm: 2,000 I.U. vitamin A and 400 I.U. vitamin D) 50 gm (1.27%), riboflavin 75 mg, Ca-pantothenate 150 mg, choline chloride 2.5 gm.

EXPERIMENTAL

In the first experiment 6 rats were depleted. They were then separated into two groups of three animals each. During the repletion period the carbohydrate and protein constituents of the diet were fed separately to the animals of group A, while group B received them simultaneously. The

results of this experiment are condensed in figure 1 and table 2. They demonstrate that the N retention of the rats which received protein and sugar simultaneously was in all cases considerably higher than that of the rats which received these two food substances separately.

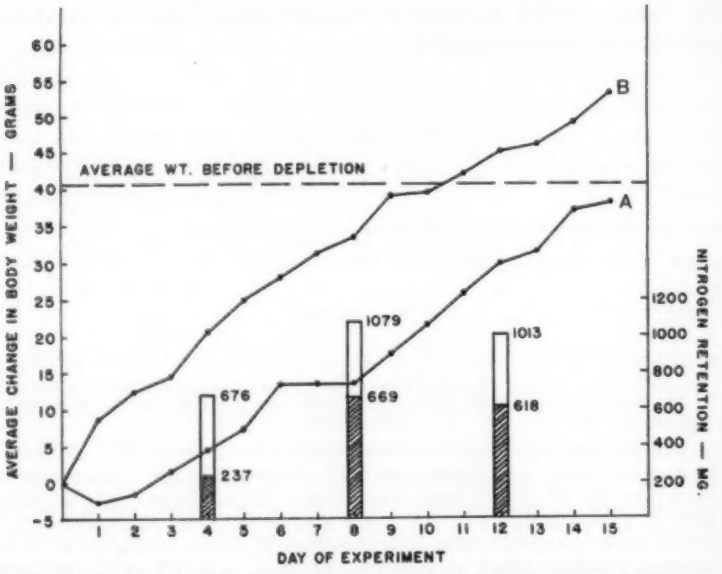

Fig. 1 Growth of rats on separate (A) and simultaneous (B) feeding of protein and sugar. Shaded column indicates N retention during the preceding 4-day period for group A, the empty column N retention for group B. The average weight of the rats before depletion was 258 gm. The ordinates on the left represent the change from the average depleted weight.

The second experiment, performed with 6 other rats, was in all essentials a repetition of the first. In this series, however, the determination of N excretion was omitted and only daily changes of body weight were registered.

During the depletion period in this experiment the rats of group A lost an average of 53.3 gm and those of group B, 51.6 gm. In the repletion period the rats in group A, which

TABLE 2

N balance during repletion of rats on separate (A) and simultaneous (B) feeding of glucose and protein (each period of 4 days duration)

GOUP RAT NO.		PERIOD I N in			PERIOD II N in			PERIOD III N in			N RETAINED IN PERIODS I–III	BODY WEIGHT		
		Urine	Feces	Retained	Urine	Feces	Retained	Urine	Feces	Retained		Starting	Depleted	Final
		mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*gm*	*gm*	*gm*
A	1	1,204	128	18	965	159	226	1,100	110	140	384	278	227	258
A	2	1,086	123	141	1,037	112	201	1,045	82	223	565	218	175	208
A	3	1,125	147	78	970	138	242	975	120	255	575	256	195	244
Total				237			669			618	1,524			
B	4	910	84	356	845	127	378	924	150	276	1,010	254	202	258
B	5	1,030	163	157	937	112	301	828	121	401	859	256	201	256
B	6	1,012	175	163	828	122	400	850	164	336	899	286	231	280
Total				676			1,079			1,013	2,768			

received sugar and protein at separate times, regained an average of 12.3 (11, 15, 11) gm in the first 14 days, and the rats of group B, receiving protein and sugar simultaneously, 46.6 (40, 57, 43) gm in the same time.[2]

The results were, therefore, essentially identical with those reported for experiment 1, demonstrating that protein-depleted rats regain their weight faster if carbohydrate is fed simultaneously with protein.

In further experiments the effect of simultaneous and separate feeding of sugar and protein was investigated with the *same* group of rats. These experiments also differed from the foregoing experiments in the composition of the diet employed. The daily protein intake was increased in experiments 3 and 4 from the earlier 2.5 gm to 3 gm casein, and, in experiment 4, the butter in the diet was increased to 4 gm instead of 2.8 gm.

In experiment 3 the three depleted rats received, during the first 8 days, diet I in the morning and diet V in the afternoon; i.e., protein and sugar were fed separately. In the following 10 days diet III was fed in the morning and diet VI in the afternoon, so that in this period the two food constituents under investigation were consumed simultaneously. Table 3 shows an increase in the daily N retention as well as daily growth rate during the second period, in which sugar and protein were fed simultaneously.

In experiment 5 the feeding periods were reversed. During the first 8 days, sugar and protein were fed simultaneously; diet VIII was offered in the morning and diet VI in the afternoon. In the following 10 days, sugar and protein were separated by feeding diet VII in the morning and diet V in the afternoon.

Table 3 demonstrates that N retention and growth were higher in the period of simultaneous feeding of protein and carbohydrate. Due to an increase of the caloric intake by

[2] In these experiments 6 hours elapsed between the end of the morning and the start of the afternoon feeding.

TABLE 3

Daily average N retention and growth on separated or simultaneous feeding of sugar and protein

(Bold faced figures represent periods of simultaneous feeding)

EXPERIMENT NO.	RATS	AVERAGE DAILY N RETENTION PERIOD		AVERAGE DAILY INCREASE OF BODY WEIGHT PERIOD	
		I	II	I	II
		mg	*mg*	*gm*	*gm*
3.	1	7	**87.5**	— 0.2	**2.4**
	2	65	**93.6**	1.6	**2.5**
	3	26	**85.5**	0.9	**3.2**
	Group average	32.7	**88.9**	0.8	**2.7**
4.	1	**88.2**	88.8	**3.9**	2.2
	2	**94.0**	63.9	**4.2**	1.7
	3	**93.8**	69.8	**4.2**	2.0
	Group average	**92.0**	74.2	**4.1**	2.0

feeding 4 gm instead of 2.8 gm of butter, growth in this experiment was generally faster than in the foregoing ones.

DISCUSSION

The experiments discussed above show that simultaneous feeding of protein and carbohydrate increases nitrogen retention and the rate of growth in protein-depleted adult rats. The effect was not restricted to a period of only two or three days, as was found by earlier authors using well-fed animals (Larson and Chaikoff, '37; Munro, '49), but has been demonstrated for extended periods of at least 14 days, i.e., until repletion of the rats was complete. These results are in agreement with those of earlier experiments on growing rats, where it was found (Geiger, '48) that feeding the dietary protein and carbohydrate at separate times resulted in impaired growth for a period of at least 21 days, the duration of the experiment.

These results support the contention that the dietary nitrogen-containing compounds which were retained when fed

simultaneously with carbohydrate participate in normal protein metabolism and are not used only for the synthesis of "stored" proteins. In normal animals the sparing action is limited because of the restricted use of excess nitrogen. In growth and repletion, however, the tissues draw steadily from all possible storage places or — *sit venia verbis* — from the "metabolic pool."

As to the mechanism of the N-sparing effect, it has been assumed (Geiger, '50) that carbohydrates may provide precursors for some missing non-essential amino acids which can be optimally utilized for protein synthesis only if fed simultaneously with the other amino acids. However, experments with labeled sugar (Geiger, Wick and Bancroft, '50) do not support this possibility.

Another possible explanation was that carbohydrate feeding increases the insulin production temporarily, and thus induces an increased retention of simultaneously fed amino acids. Experiments on diabetic rats (Geiger, Wick and Bancroft, '50)do not support such an assumption, either.

It seems conceivable, therefore, that carbohydrates carried to the liver cells through the portal circulation have some specific physiological function which enables them to expedite the utilization of dietary amino acids. Acetylation, phosphorylation or some other intermediary compound-formation may possibly be involved. The recent experiments of Miller et al. ('50) on the utilization of labeled lysine by the isolated, perfused liver support such a hypothesis. Experiments on this problem are in progress in our laboratories.

SUMMARY

1. Using protein-depleted adult rats, the effect of the time factor in the feeding of protein and carbohydrate has been investigated. It was found that the N retention and growth of the rats which received protein and sugar simultaneously were in all cases considerably higher than those of the rats which received these two food substances separately.

2. The protein-sparing and growth-promoting effects of simultaneous feeding have been demonstrated for a period of 14 days.

3. The biological significance of these results is discussed.

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NUTRITIONAL VALUE OF PLANT MATERIALS

V. REPRODUCTION OF RATS FED PURIFIED RATIONS CONTAINING SOYBEAN PROTEIN [1]

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In a previous paper (Schultze, '50a) it was reported that 4 generations of rats maintained on a purified ration containing a commercial soybean protein and DL-methionine as the only source of amino acids made satisfactory postweaning weight gains, and that the postweaning mortality of these animals was very low. The addition of liver extract, or fish solubles or the subcutaneous injection of vitamin B_{12} did not increase the weight increments of the rats, nor did purification of the soybean proteins impair their growth-promoting properties. The present paper summarizes observations on the reproductive performance of rats maintained on these rations. The literature does not appear to contain data on the reproductive performance of animals maintained on purified rations containing isolated plant proteins. An excellent summary of available information on the diet of laboratory animals in relation to production and the viability of the young has recently been published by Russell ('48).

EXPERIMENTAL

The ingredients and composition of the rations were those described in paper I of this series (Schultze, '50a). Summarized briefly, rations S_1 and S_2 contained sucrose, hydro-

[1] Paper 2575, Scientific Journal Series, Minnesota Agricultural Experiment Station.

genated vegetable fat, corn oil, salts, and a commercial soybean protein preparation [2] supplemented with DL-methionine, whereas in ration S_3 this protein had been purified by two reprecipitations from an aqueous medium, followed by extensive extraction with boiling alcohol. The protein content of the rations was 24%. The vitamin supplement to rations S_2 and S_3 contained all available vitamins except ascorbic acid and vitamin B_{12}; in ration S_1 biotin was not included. In ration C_1 alcohol-extracted casein replaced the plant protein and DL-methionine of ration S_1.

The animals were among those used for the growth experiments which were conducted as a part of this series (Schultze, '50a). The females were bred when 10 weeks old with males maintained on the same rations. Shortly before parturition and during lactation the females were housed individually in cages containing clean wood shavings. The young were weaned when 21 days old. In order to subject the females to the maximum challenge of reproductive performance, the size of the litter was never reduced and the females were returned to the breeding cages immediately after weaning or after the loss of a litter. No attempt was made in this study to determine the length of the reproductive effectiveness of these rats. The rats which were fed the casein ration were F_3 generation offspring of mothers maintained on the same ration.

RESULTS AND DISCUSSION

The data on postparturient reproductive performance are summarized in tables 1 and 2. The most striking effect of the rations containing the commercial soybean protein was the high incidence of mortality of the young during the first 4 days of life (groups 2–7, 9). Most of these early deaths were preceded by symptoms of "acute uremia of the newborn." This syndrome has been described elsewhere with respect to symptomatology and prevention by vitamin B_{12}

[2] Archer-Daniels-Midland Co., Minneapolis.

TABLE 1

Reproduction of rats on purified rations

GROUP	1	2	3	4	5	6
Generation on soybean protein	..	Parent	F_1	F_2	F_3	F_1 and F_2
Ration	C_1	S_1	S_1	S_1	S_1	S_2
Source of protein	Casein	Commercial Soybean protein				
Number of females	43	26	55	22	19	38
Number of sterile females	2	0	3	2	1	4
Number of pregnancies	91	96	142	64	51	70
Number of litters born alive	89	88	136	58	48	69
Mean number of young born per litter	6.94	6.40	6.55	5.61	6.73	6.90
Per cent of young born dead	4.2	7.6	4.1	11.0	9.0	9.5
Mortality of young, 0–4th day (%)	10.7	41.9	49.7	54.6	46.2	53.8
Mortality of young, 0–21st day (%)	17.6	55.0	59.0	62.5	52.7	56.5
Mean number of young weaned per litter of survivors [1]	6.45	5.67	5.31	5.06	6.05	6.22
Mean 21-day weight per survivor	31.65	34.80	35.46	34.34	35.34	33.49
± Std. error of mean	0.22	0.33	0.39	0.33	0.39	0.30

[1] Litters in which all young died were not included in calculating these values.

TABLE 2

Effect of supplementation and purification of soybean protein on reproduction of rats

GROUP	7	8	9	10	11	12	13	14
	Litter mates		Litter mates		Same rats		Same rats	
					1st–3rd Litters	3rd–5th Litters	1st Litters	2nd Litters
Generation on soy protein	F_1	F_1	F_1	F_1	Parent, F_1	Parent, F_1	F_2, F_3	F_2, F_3
Ration	S_1	S_1	S_1	S_1	S_1	S_1	S_2	S_2
Supplement	None	2% L.E.[1]	None	3% fish sol.	None	2% L.E.[1]	None	None
Source of protein	Commercial soybean protein						Purified soybean protein	
Number of females bred	6	7	19	21	14	14	18	16
Number of sterile females	0	0	1	1	0	0	0	0
Number of pregnancies	17	21	44	44	37	19	18	16
Number of litters born alive	16	20	42	44	37	18	18	16
Mean number of young born per litter	5.31	8.14	7.04	7.41	6.00	7.90	8.70	7.90
Per cent of young born dead	9.6	5.1	4.2	0.6	3.0	10.0	7.7	11.4
Mortality of young, 0–4th day (%)	68.2	0.0	33.6	7.6	94.9	9.6	7.6	10.3
Mortality of young, 0–21st day (%)	72.9	13.8	47.4	21.2	96.7	34.8	18.7	50.4
Mean number of young weaned per litter of survivors[2]	5.67	5.82	6.00	6.70	2.33	5.70	6.88	5.09
Mean 21-day weight per survivor	34.00	38.25	36.12	34.67	30.10	41.37	33.03	33.84
± Std. error of mean	1.15	0.41	0.40	0.27	2.20	0.72	0.32	0.53

[1] 1:20 Liver extract, Wilson.
[2] Litters in which all young died were not included in calculating these values.

(Schultze, '49) or by substances of animal origin, as well as its incidence among young born to mothers fed purified or natural plant rations (Halvorson and Schultze, '50). In groups 2–7 and 9, from 30 to 40% of the litters died with the typical symptoms of acute uremia of the newborn. On rations S_1 and S_2 there was never any evidence that insufficient quantities of milk secreted by the mothers were responsible for the high mortality, nor was there any indication that the mortality of the young increased with successive generations. The rations used maintained the lactating females in good condition. Thus the mean weight losses during lactation in two groups of 16 rats each, which were fed rations S_2 and S_3 and which raised to weaning age 7.1 and 8.1 young per litter, respectively, were only 0.5 ± 2.7 gm [3] and 5.4 ± 2.5 gm.

Among the 518 pregnancies of rats on the unsupplemented rations, evidence was obtained for only 8 cases of resorption of complete litters; these occurred in groups 2, 3, 4 and 6. No cases of toxemia of pregnancy, as observed by Spitzer and Phillips ('46) in rats fed corn-soybean-alfalfa rations, were encountered in these experiments. Most of the young which survived reached satisfactory weaning weights in all generations. The addition of biotin to ration S_1 did not improve the reproductive performance (group 6). The high mortality of the young born to mothers fed ration S_1 could be greatly reduced by the addition to this ration of either 2% liver extract [4] or of 3% condensed fish solubles, as is shown in table 2. Inasmuch as these same supplements prevented the occurence of acute uremia of the newborn (Halvorson and Schultze, '50), the higher rate of survival can be satisfactorily accounted for by the elimination of this syndrome in the young through better nutrition of their mothers. The decisive effect of maternal nutrition on the early survival of the young is particularly well illustrated in the data for groups 11 and 12. Group 11 was made up of females selected from groups 2 and 3 because of consistent failure to raise their young.

[3] Standard error of mean.
[4] Wilson's 1:20.

When these rats were subsequently fed the ration containing 2% liver extract throughout pregnancy and lactation, the survival of the young, particularly during the first few days, was greatly improved (group 12).

Rations S_1, S_2 and S_3 used in these experiments were presumably low in vitamin B_{12}: in particular ration S_3, the protein of which had been extracted with three changes of boiling alcohol for a total of 72 hours. The females in groups 13 and 14 had been fed ration S_3 since weaning and they were F_2 or F_3 generation progeny of rats continuously fed the plant ration S_1. Their first litters had a very low mortality, suggesting that under a suitable dietary regime satisfactory reproduction of the rat can be obtained with rations very low in, or devoid of, vitamin B_{12}. Lepkovsky et al. ('50) have recently suggested that "vitamin B_{12} deficiency interfered with lactation less than with gestation" of rats fed from the time of mating on a high protein ration containing vegetable seeds. Emerson et al. ('50) reported that the weaning weights of rats born to mothers fed rations containing soybean meal could be increased by the daily feeding of 2.5 μg of vitamin B_{12} to both mothers and offspring.

The second litters (group 14) of the rats maintained since weaning on the purified soybean protein ration S_3 had a much higher mortality and, like the first litters, a very low incidence of acute uremia of the newborn. Emerson et al. ('50) have recently suggested that there is a gradual depletion of factors essential for the normal reproduction of female rats when they are maintained on rations containing plant proteins. An extension of our experiments with the purified ration S_3 into subsequent generations supports the view that a gradual depletion of the maternal organisms was operative in the greatly increased mortality of the second litters (group 14) as compared to the first litters (group 13) produced by the same mothers. These experiments will be reported later.

A comparison of the data reported for groups 7 and 11 with those for groups 8 and 12, respectively, indicated that

feeding liver extract to the mothers may increase the number of young born. Since this suggested effect of liver extract may be due to a variety of causes operative during the preparturient reproductive phase, the observations summarized in table 3 were made. The matched litter-mate females used for this experiment were F_2 generation progeny of parents fed ration S_1. From the 6th week of life one group was fed ration S_2, while the other received the same ration plus 2% liver extract. The rats were killed on the 21st day of the first pregnancy and counts were made of

TABLE 3

Preparturient reproductive performance

CATEGORY OF INTEREST	RATION S_2	
	Without liver extract	With 2% liver extract
Number of females (litter mates)	20	21
Mean length of 74 estrous cycles (days)	5.59 ± 0.53 [1]	5.63 ± 0.54 [1]
Weight of females, 21 days pregnant (gm)	233.3 ± 4.1 [2]	247.8 ± 5.0 [2]
Weight of females after removal of young (gm)	191.0 ± 4.1	202.4 ± 4.6
Age of females at term (days)	97.4 ± 0.9	100.0 ± 1.7
Mean number of corpora lutea	10.0 ± 0.26	9.81 ± 0.27
Mean number of implants	8.80 ± 0.46	9.19 ± 0.34
Mean number of resorption sites	0.90 ± 0.26	0.48 ± 0.15
Mean number of live young	7.90 ± 0.37	8.71 ± 0.35
Mean weight per fetus removed (gm)	4.24 ± 0.03	4.24 ± 0.02

[1] Standard error of mean.

[2] Significant difference at P = 0.05.

corpora lutea, implantation sites, resorptions and fetuses. While the number of young born to the females consuming ration S_2 fortified with liver extract was higher and the number of resorptions was lower than in the unsupplemented group, these differences were not significant statistically ($P = > 0.05$). The only difference between these two groups of rats which was statistically significant ($P = < 0.05$) was the weight of the females on the 21st day of pregnancy. The high number of young born in groups 13 and 14, which were maintained on the purified soybean protein ration S_3, in-

dicates also that normal pregnancy can take place in females consuming rations very low in vitamin B_{12} activity. Whether these young were normal functionally or whether defects established *in utero* contribute to the high mortality observed in group 14 cannot be decided on the basis of the evidence presented here. Dryden et al. ('49) have reported, however, that vitamin B_{12} deficiency can lead to a decreased size of the ovaries and uteri of rats.

The possibility that microbiological synthesis in the intestine supplied these rats with vitamin B_{12} and perhaps with other essential factors must be considered in view of the observations reported by Hartman et al. ('49) and by Emerson et al. ('50). Other studies made in this laboratory (Schultze, '50b) have shown that the addition of 2% phthalylsulfathiazole [5] to ration S_2 did not interfere with the reproductive performance of the rats. In fact, the survival of the young born to mothers consuming the bacteriostatic compound was improved, and no cases of acute uremia of the newborn were observed. Inasmuch as the incidence of acute uremia on the unsupplemented ration S_2 was high, the synthesis of vitamin B_{12} by the intestinal microflora was apparently not very extensive, and it may have been increased as a result of changes in the intestinal microflora caused by prolonged administration of the sulfa drug. It is possible that microbiological synthesis of vitamin B_{12} accounts in part for the very low incidence of the external symptoms of acute uremia of the newborn observed in groups 13 and 14 and in extensive unpublished experiments with other rats fed the same ration. Zucker and Zucker ('50) have reported that the feces of rats fed an all-plant ration containing cottonseed meal were persistently negative for vitamin B_{12}, despite the feeding of a high level of riboflavin.

SUMMARY

1. Rats fed from weaning purified rations containing 24% protein in the form of a commercial soybean protein and

[5] Sharp and Dohme, Inc.

DL-methionine produced young which had a very high mortality due to acute uremia of the newborn. The addition of liver extract or condensed fish solubles to the ration of the mothers greatly increased the rate of survival of the young. The survivors in the litters on the unsupplemented rations reproduced for two subsequent generations with essentially the same rate of mortality.

2. Rats fed rations in which the commercial soybean protein had been extensively purified produced first litters which had a relatively low mortality. In the second litters the mortality of the young was much increased.

3. The addition of liver extract to the ration containing the commercial soybean protein had no significant effect on the length of the estrous cycle or on the number of corpora lutea, implants, and resorptions, or the number or weight of young produced in the first pregnancy.

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EFFECT OF STEROLS ON THE GROWTH OF CHICKS FED HIGH ALFALFA DIETS OR A DIET CONTAINING QUILLAJA SAPONIN

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That chicks manifest a distinct dislike of alfalfa meal when given a free choice of feedstuffs has been observed by Graham ('32), Dove ('35), and Jukes ('38). The cause of the unpalatability of alfalfa meal has not been explained. It has been demonstrated, however, that high levels of alfalfa meal in the diet of young chicks cause a depression of growth which is proportional to the level in the ration (Draper, '48; Cooney et al., '48; Lepkovsky et al., '50). Concentrates can be obtained from alfalfa meal which inhibit the growth of chicks when fed in the ration. The properties of these extracts suggest that the inhibitory effect of high levels of alfalfa meal may be due to the presence of saponins. The feeding of cholesterol in the diet counteracts, to a large extent, the growth-depressing effect of alfalfa meal or of the inhibitory extracts (Peterson, '50; Kodras et al., '50).

The present report describes further investigations on the relation of dietary sterols to the growth of chicks fed either a high alfalfa diet or a diet containing *Quillaja* saponin.

METHODS

Growth tests were made on 7- to 14-day-old Single Comb White Leghorn chicks that had previously been maintained on

a standard stock diet. The chicks were selected for uniformity of weight and rate of growth and maintained on the experimental diets for periods of 21 days, except in instances where lack of special ingredients required an earlier termination of the experiment.

The composition of the basal diet was identical with that employed in previous studies (Peterson, '50). It consisted of the following ingredients: ground barley 30, ground corn 22, ground wheat 10, wheat bran 10, soybean oil meal 10, fish meal 7.5, dried whey 2.5, dried skim milk 2.5, liver meal 2, ground limestone 1.5, steamed bonemeal 1.2, sodium chloride 0.5, $MnSO_4 \cdot H_2O$ 0.025, fortified fish oil (2250 A–400 D per gm) 0.25 gm per 100 gm of diet, and riboflavin 2 mg per kilogram. Ingredients employed in the experimental diets were substituted for a like amount of barley in the basal diet. The high alfalfa diet used in these experiments contained 20% dehydrated alfalfa meal, which was substituted for an equal amount of barley.

In some of the experiments the cholesterol content of plasma was determined by the method of Schoenheimer and Sperry as modified by Hawk, Oser and Summerson ('47).

EXPERIMENTAL AND RESULTS

Earlier experiments had shown that the addition of cholesterol to a ration containing 20% alfalfa meal largely counteracted the growth-depressing effect ordinarily produced by this diet. An experiment was designed to determine whether this effect would be enhanced by the addition of extra fat to the diet, since cholesterol is more readily absorbed in the presence of fat. Three levels of cholesterol (0.5%, 1.0%, and 1.5%) were used. At each level cholesterol was added to a 20% alfalfa diet with and without the inclusion of 4% cottonseed oil.[1] The cholesterol was dissolved in the oil by warming. After cooling to room temperature, the thick

[1] Wesson oil.

paste which resulted was mixed with the alfalfa meal and this mixture added to the remainder of the diet.

At the conclusion of the growth test samples of plasma were taken for analysis from 6 of the 9 chicks in each group. Blood samples from two chicks were pooled so that three samples represented 6 chicks in each group. Growth results and plasma cholesterol are shown in table 1.

TABLE 1

Effect of cholesterol and cottonseed oil on the growth of chicks fed 20% alfalfa diets
(12-day-old chicks, 9 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 21 DAYS	AVERAGE TOTAL PLASMA CHOLESTEROL [1]
	gm	*mg/100 ml*
Basal diet	194	64
20% alfalfa	123	58
20% alfalfa, 4% cottonseed oil	146	73
20% alfalfa, 0.5% cholesterol	167	118
20% alfalfa, 0.5% cholesterol, 4% cottonseed oil	191	210
20% alfalfa, 1.0% cholesterol	180	211
20% alfalfa, 1.0% cholesterol, 4% cottonseed oil	200	531
20% alfalfa, 1.5% cholesterol	172	196
20% alfalfa, 1.5% cholesterol, 4% cottonseed oil	222	594

[1] Average value for 6 chicks in each group.

The growth-depressing effect of alfalfa meal was completely overcome by the inclusion of both cholesterol and cottonseed oil in the diet. Cholesterol was not quite as effective alone as in the presence of additional fat.

The data shown in table 1 might appear to suggest a relationship between plasma cholesterol level and prevention of growth depression on the high alfalfa diets. Subsequent experiments, however, showed that optimum growth on a high alfalfa diet need not necessarily be associated with elevated plasma sterols.

Estrogen treatment

If elevation of the blood cholesterol were the only factor involved, treatment with estrogens might possibly be expected to have an effect similar to that of ingestion of cholesterol. Implantation of estrogens produces a marked increase of blood lipids, including cholesterol, in the chick (Entenman et al., '40). Two-week-old chicks were divided into 4 groups of 25 each. Two groups were placed on the 20% alfalfa diet and all the chicks of one of these groups were given subcutaneous implants of 15 mg of diethylstilbestrol. The remaining two groups were placed on the basal diet and each chick in one of these groups was also implanted with diethylstilbestrol.

TABLE 2

Effect of diethylstilbestrol implants on growth of chicks on high alfalfa diets

(14-day-old chicks, 25 chicks per group)

TREATMENT	AVERAGE GAIN IN WEIGHT, 21 DAYS
	gm
20% alfalfa	145
20% alfalfa (diethylstilbestrol)	121
Basal	172
Basal (diethylstilbestrol)	192

Growth results in this trial (table 2) indicated that the estrogen treatment was ineffective as a means of preventing the growth-depressing effect of a high alfalfa diet. The chicks on the control diet grew normally, indicating no growth-depressing effect from diethylstilbestrol itself.

It appeared that the diethylstilbestrol treatment produced a slight growth depression in the group on the alfalfa diet. In a later experiment (table 5) where 2% cottonseed oil was added to the alfalfa diet, the growth depression was even more marked in the group given the estrogen treatment, even though plasma cholesterol was markedly increased.

Phytosterols

If the effectiveness of the sterol were not dependent upon intestinal absorption, it might be expected that phytosterols would be as effective as cholesterol. Phytosterols are not absorbed by mice (Schoenheimer, '31; Sperry and Bergmann, '37; Breusch, '38). It might be anticipated that this would also be true of the chick. Diets were prepared containing mixed soy sterols [2] both with and without the addition of cottonseed oil. The results of this test (table 3) indicate that the soy sterols were effective in counteracting the alfalfa growth inhibitor. A comparison of growth results between a cholesterol-alfalfa diet and a soy sterol-alfalfa diet (table 4) shows that both were equally effective. Table 4 shows the effect of varying the levels of cholesterol and of the soy sterols. Table 5 shows the effect of varying the level of cottonseed oil.

Analysis of the plasma (table 4) demonstrated that total plasma sterols (calculated as cholesterol), determinable by the Liebermann-Burchard reaction, were not increased by the addition of the phytosterol mixture to the diet. This demonstrates that the sterol content of the plasma is not a critical factor in preventing growth depression by alfalfa meal. These results would also appear to suggest that chicks, like mice, may have a capacity for selective absorption of cholesterol and that there is limited absorption of phytosterols. The mixed soy sterols were found to give a Liebermann-Burchard color reaction similar in intensity to that produced by cholesterol itself.

In table 5 are also shown the results of feeding several sterol fractions obtained from tall oil. Tallene [3] contains 9.0 to 10.5% sterol as determined by the digitonin analysis. Tallene ester [4] contains approximately 22% sterol. The Tal-

[2] Mixed soy sterols supplied by Distillation Products, Inc., contained a minimum of 95% sterols, principally sitosterol and stigmasterol.

[3] Samples and proximate analyses supplied by Dr. Paul R. Wiley of the West Virginia Pulp and Paper Company. The principal sterol is probably B-sitosterol.

[4] See footnote 3.

TABLE 3

Effect of soy sterols on the growth of chicks fed a 20% alfalfa diet

(7-day-old chicks, 8 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 21 DAYS
	gm
Basal diet	153
20% alfalfa	106
Stock diet	153
20% alfalfa, 1% soy sterols	138
20% alfalfa, 1% soy sterols, 4% cottonseed oil	148

TABLE 4

Effect of various levels of sterols on growth and plasma cholesterol levels

(8-day-old chicks, 10 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 21 DAYS	TOTAL PLASMA CHOLESTEROL [1]
	gm	*mg/100 ml*
Basal diet	182	73
20% alfalfa	116	73
20% alfalfa, 4% cottonseed oil	152	72
20% alfalfa, 4% cottonseed oil, 0.1% cholesterol	148	79
20% alfalfa, 4% cottonseed oil, 0.2% cholesterol	172	102
20% alfalfa, 4% cottonseed oil, 0.5% cholesterol	187	205
20% alfalfa, 4% cottonseed oil, 1% soy sterols	191	70
20% alfalfa, 4% cottonseed oil, 0.5% soy sterols	190	67
20% alfalfa, 4% cottonseed oil, 0.2% soy sterols	182	75

[1] Average value for 8 chicks in each group.

lene ester unsaponifiable was prepared by the author and was not analyzed for sterol content. The fractions richest in sterols both had a definite growth-promoting action on chicks fed the 20% alfalfa diet.

TABLE 5

Comparison of effecetiveness of various sterol mixtures

(11-day-old chicks, 10 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 21 DAYS	TOTAL PLASMA CHOLESTEROL [1]
	gm	*mg/100 ml*
Basal diet	183	..
20% alfalfa	146	64
20% alfalfa, 2% cottonseed oil	127	69
20% alfalfa, 2% cottonseed oil, diethylstilbestrol-treated	80	799
20% alfalfa, 4% cottonseed oil, 0.5% cholesterol	196	174
20% alfalfa, 4% cottonseed oil, 0.5% soy sterols	193	
20% alfalfa, 4% cottonseed oil, 0.2% soy sterols	188	
20% alfalfa, 3% cottonseed oil, 0.2% soy sterols	196	
20% alfalfa, 2% cottonseed oil, 0.2% soy sterols	164	
20% alfalfa, 1% cottonseed oil, 0.2% soy sterols	182	
20% alfalfa, 3% Tallene, 2% cottonseed oil	116	
20% alfalfa, 4% Tallene ester	171	
20% alfalfa, 2% unsaponifiables from Tallene ester, + 2% cottonseed oil	171	

[1] Average value for 6 chicks in each group.

Quillaja saponin

Earlier work had indicated that the growth-depressing properties of alfalfa meal may probably be explained as due to the presence of saponins in alfalfa (Peterson, '50). It appeared desirable to test the effect of cholesterol on the

TABLE 6

Effect of Quillaja saponin on growth

(10-day-old chicks, 10 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 17 DAYS
	gm
Basal	147
0.3% *Quillaja* saponin [1]	44
0.3% *Quillaja* saponin, 1% cholesterol	116
0.3% *Quillaja* saponin, 1% cholesterol, 4% cottonseed oil	146

[1] Merck. Saponin Pure.

TABLE 7

Growth results with Quillaja saponin

(7-day-old chicks, 10 chicks per group)

DIET	AVERAGE GAIN IN WEIGHT, 21 DAYS
	gm
Basal	162
Basal, 4% cottonseed oil	154
0.5% cholesterol, 4% cottonseed oil	159
0.1% saponin [1]	150
0.2% saponin	139
0.3% saponin	124
0.1% saponin, 4% cottonseed oil, 0.5% cholesterol	158
0.2% saponin, 4% cottonseed oil, 0.5% cholesterol	164
0.3% saponin, 4% cottonseed oil, 0.5% cholesterol	163
0.5% saponin, 4% cottonseed oil, 0.5% cholesterol	152

[1] Supplied by S. B. Penick and Co.

growth of chicks fed a diet containing a known saponin. Diets were prepared containing a saponin preparation from *Quillaja saponaria*.[5] The growth results on these diets were directly parallel to those obtained with the alfalfa diets: a marked growth depression was obtained with the saponin-containing diet and this depression was largely overcome by the addition of cholesterol. Addition of both cottonseed oil and cholesterol completely counteracted the growth-inhibitory action of the saponin (table 6).

[5] Merck Saponin Pure.

This experiment was repeated, using another sample of *Quillaja* saponin.[6] Although growth depression was not as marked with this preparation, improved growth was obtained on adding cottonseed oil and cholesterol (table 7).

DISCUSSION

The growth depression produced by high levels of alfalfa meal (here studied at the 20% level) is completely counteracted by the addition of cottonseed oil and cholesterol to the diet. It had previously been shown that the addition of cholesterol alone to a high alfalfa diet produced a very marked growth effect. It has been suggested that the inhibitory action of alfalfa meal may be due to the presence of saponins in alfalfa (Peterson, '50). This conclusion is strengthened, although not proved, by the fact that a similar effect can be obtained with *Quillaja* saponin.

That the effectiveness of the fat-cholesterol mixture in these diets was not due to increased caloric density of the ration is shown by the ineffectiveness of the addition of cottonseed oil alone to such an inhibitory diet. Previous experiments (Lepkovsky et al., '50) had shown that exhaustive extraction of alfalfa meal with water removed the growth-inhibitory factor or factors. Thus the depressing effect on growth could not be explained by the high fiber content of the alfalfa meal.

The action of cholesterol in preventing growth depression by alfalfa is not specific, since phytosterols will produce the same effect. It is suggested that this effect is due to the formation of an insoluble sterol-saponin compound in the digestive tract. The apparent relationship between plasma cholesterol and growth (table 1) on a 20% alfalfa diet is evidently merely an indication of the increased solubility of cholesterol in the presence of added fat. Raising blood cholesterol by means of estrogen treatment did not affect growth favorably. Addition of phytosterols to the diet counteracted the growth-depressing effect without raising the level of blood

[6] Supplied by S. B. Penick and Co.

sterols ("cholesterol") determinable by the Liebermann-Burchard reaction. These results also suggest that phytosterols are not appreciably absorbed by the chick.

SUMMARY

Addition of both cholesterol and cottonseed oil to a diet containing 20% alfalfa meal completely prevented the growth depression otherwise produced in chicks on such a diet. An identical effect was obtained with cottonseed oil and a phytosterol mixture prepared from soybeans.

The growth depression produced by *Quillaja* saponin in the chick diet was also prevented by the addition of a mixture of cottonseed oil and cholesterol to the diet.

Raising the chicks' blood level of cholesterol by treatment with diethylstilbestrol was ineffective as a means of preventing the growth depression brought about by a high level of alfalfa meal in the diet. Counteraction of the alfalfa growth inhibitor or inhibitors was not dependent upon an increase in the plasma level of sterols determinable by the Liebermann-Burchard reaction.

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THE SODIUM AND POTASSIUM REQUIREMENTS OF THE RAT FOR GROWTH[1]

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THREE FIGURES

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INTRODUCTION

An investigation into the sodium and potassium requirements of the rat for growth was prompted by the lack of agreement on the minimum amount required for optimum growth, and the absence of direct information as to the effect of one element on the requirement for the other. The requirement of the rat for sodium was found to range from 0.035% (Osborne and Mendel, '18) to 0.3% (St. John, '28), with additional values reported between these two extremes (Miller, '23; Olson and St. John, '25).

The potassium requirement was established at 15 mg per rat per day (0.15% on the basis of a food consumption of 10 gm per day) by Miller ('26b). More recently, Kornberg and Endicott ('46) stated that 0.13% potassium was the amount required to prevent the occurrence of the typical symptoms of potassium deficiency. This figure was increased to 0.17% before optimum growth resulted.

The effect of one element on the requirement for the other had been investigated by indirect means. Bunge (1873), on the basis of experiments conducted upon himself and the ob-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the American Salt Manufacturers' Association.

served high intake of salt by cattle, advanced the theory that a high potassium intake increased the requirement for sodium. This theory has been generally accepted, but data have been offered both for (Gerard, '12; Glantzel, '34) and against it (Miller, '26a; Hart, McCollum, Steenbock and Humphrey, '11). When the rat was used as the experimental animal (St. John, '28), it was concluded that the absolute amounts of sodium and potassium were important, rather than the ratio of one element to the other. More recently the effect of sodium on the potassium requirement of the lactic acid bacteria has been studied (MacLeod and Snell, '48). The requirement for potassium was found to be dependent on the sodium concentration of the medium; the greater the amount of sodium in the medium the greater the requirement for potassium.

The lack of agreement as to the sodium requirement (0.035 to 0.3%), the absence of direct evidence of an effect of sodium or potassium upon the requirement for the other, and the current availability of highly purified rations warranted a detailed investigation for the purpose of determining the sodium and potassium requirements of the rat and their interrelationships.

EXPERIMENTAL

The basal ration, which contained 0.005% sodium and 0.005% potassium by flame photometric analysis, was that used by Grunert and Phillips ('49). The sodium and potassium were added in the form of their bicarbonates at the expense of the whole ration, as is indicated in figures 1, 2 and 3.

Male weanling rats (Holtzman), weighing 40 to 45 gm, were placed in raised wire-screened individual cages for an experimental period of 6 weeks. The feed was supplied ad libitum in conical cups and the distilled water in inverted brown bottles to which glass nipples were attached. The animals were weighed weekly and observed for symptoms of sodium or potassium deficiencies and respiratory infections. Those animals which exhibited respiratory infections were removed from the experiment.

Ten to 11 rats were used for each test level of sodium or potassium except for the two lowest levels of sodium (0.005 and 0.01%), the lowest level of potassium (0.06%) and the series in which 1.0% potassium was used. In these groups 5 rats were used.

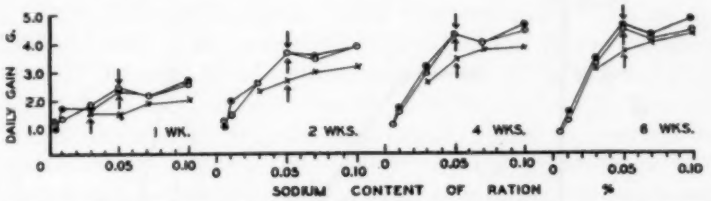

Fig. 1 The sodium requirement of the rat for growth, as affected by potassium and the duration of the experiment. A level of 0.25% potassium is represented by a dot, 0.5% by a circle and 1.0% by a cross. The requirement for sodium on each level of potassium is indicated by an arrow.

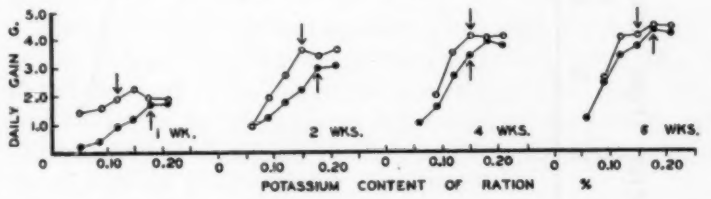

Fig. 2 The potassium requirement of the rat for growth, as affected by sodium and the duration of the experiment. A level of 0.1% sodium is represented by a dot and 1.0% by a circle. The requirement for potassium on each level of sodium is indicated by an arrow.

The data were analyzed statistically. The minimum requirement was established as the lowest level of sodium or potassium at which the growth rate was not significantly different ($P > 0.01$) from the maximum growth rate obtained at any level.

RESULTS

(a) Sodium requirement

The effect of potassium on the sodium requirement of the rat for growth was determined by feeding 5 levels of sodium,

between 0.005 and 0.1%, in the presence of three levels of potassium (0.25, 0.5 and 1.0%). The results, presented in 4 time intervals of one, two, 4 and 6 weeks' duration, are shown in figure 1.

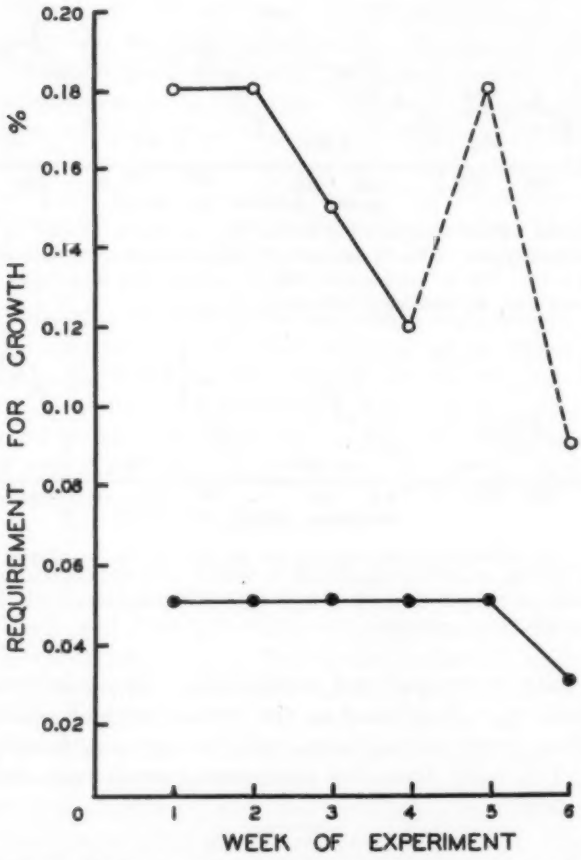

Fig. 3 The sodium and potassium requirements of the rat for growth, during each week of a 6-week experimental period. The potassium requirement (in the presence of 0.1% sodium) is represented by a circle. The sodium requirement (in the presence of 0.25% potassium) is represented by a dot.

It was observed that the rate of growth increased progressively as the sodium in the ration was increased from 0.005 to 0.05%. When higher levels of sodium were present (0.07 and 0.1%), the rate of growth failed to increase further. The sodium requirement of the rat for growth was therefore established at 0.05%.

As is indicated in figure 1, the requirement for sodium was independent of the potassium content of the ration. Regardless of the amount of potassium present (0.25, 0.5 or 1.0%), the sodium requirement remained constant at 0.05%. The only exception was during the first week, when the sodium requirement was decreased to 0.03% by the presence of 1.0% potassium, which is a toxic level of potassium. It was noted, however, that the decrease in requirement was due to a depression of the growth rate, in the presence of this level of potassium, at the higher levels of sodium (0.05, 0.07 and 0.1%).

Although the potassium content of the ration had no effect on the sodium requirement of the rat, it was noted (fig. 1) that a level of 1.0% potassium was toxic. The toxic effect was evidenced by the general depression of the growth rate at all the levels of sodium studied, an effect most evident during the initial phases of the experiment.

(b) Potassium requirement

The effect of sodium on the potassium requirement of the rat for growth, was determined by feeding 6 levels of potassium, between 0.06 and 0.21%, in the presence of two levels of sodium (0.1 and 1.0%). The results are graphically presented in figure 2 for time intervals of one, two, 4 and 6 weeks' duration.

In the presence of 0.1% sodium a maximum rate of growth was obtained with 0.18% potassium at all the time intervals studied except that of 4 weeks, when the potassium requirement dropped to 0.15%. Due to the large within-group variation at this time interval, the growth rate on the 0.15% level was not significantly different from that on the 0.18% level.

Despite this discrepancy it was apparent that the potassium requirement of the rat for growth was 0.18% in the presence of 0.1% sodium.

The level of dietary sodium affected the potassium requirement. When the sodium content of the ration was increased from 0.1 to 1.0%, the requirement for potassium dropped from 0.18 to 0.12% at the end of the first week and remained at 0.15% for the two- 4- and 6-week intervals. The decrease in the potassium requirement occasioned by high sodium intake was demonstrated with critical levels of potassium (0.12 and 0.15%). Hence the potassium requirement was lowered from 0.18 to 0.15% by a 10-fold increase in the sodium content of the ration (0.1 to 1.0%). The sparing action of sodium, when the potassium was limiting, was evident to the greatest degree during the first week. It then decreased progressively as the time interval of the experiment was lengthened until it was almost absent.

It was significant that the sparing action of sodium at critical potassium levels disappeared as the experiment progressed, and eventually a potassium deficiency developed when the potassium content of the ration was sufficiently low. When 0.06% potassium was present, the 10-fold increase in sodium (1.0%) resulted in a marked improvement in the rate of growth during the first week only. This was halted by the end of the second week, and during the 4th week all the rats which received 1.0% sodium with 0.06% potassium showed the diarrhea and distended abdomens typical of a potassium deficiency (Thomas, Mylon and Winternitz, '39–'40; Follis, Orent-Keiles and McCollum, '42). Three of the 5 rats died, with visible heart lesions observable on autopsy. One more rat in this group died by the 6th week. The necessity for a minimum amount of potassium in the presence of an excess of sodium was thus clearly demonstrated. The minimum potassium required in the presence of 1.0% sodium was found to be 0.09%.

When the growth data for the sodium and potassium requirements were examined by weeks it was noted that the

potassium requirement steadily declined, while the sodium requirement remained constant or nearly so (fig. 3). The requirement for sodium remained constant at the 0.05% level for the first 5 weeks of the experimental period but was reduced to 0.03% during the 6th week. In contrast, the potassium requirement remained constant at 0.18% for the first two weeks. It then dropped progressively to 0.15 and 0.12% in the third and 4th weeks, and finally to 0.09% in the 6th week. The increased requirement exhibited by the data of figure 3 during the 5th week was due to an extraordinarily high rate of growth during this period.

DISCUSSION

That the sodium requirement of the rat for growth differs from that for potassium is evident from the data presented and is shown not only in the absolute amounts required but also in the effect of one element on the requirement for the other. The sodium requirement of 0.05% was independent of the potassium in the ration, even when the latter was increased 4-fold. This may be considered evidence against the hypothesis of Bunge (1873) that a high potassium intake necessitated an increased intake of sodium.

In contrast, the potassium requirement was influenced by the sodium content of the ration. This effect was most evident during the first week, became less evident as the experiment progressed, especially at the lower levels of potassium, and was absent at the end of the experimental period of 6 weeks. The decrease in the potassium requirement occasioned by the high level of sodium (1.0%) was due to the sparing action of sodium favoring the growth rate of the rats fed critical amounts of potassium (less than 0.18%). The mechanism whereby a high level of sodium improved the growth rate of rats fed subminimum levels of potassium is not clear at present, but may involve a shift of the two ions between the intra- and extracellular phases of the body. It is felt that an explanation lies in either the

replacement of intracellular potassium by sodium or the prevention by the excess sodium of the loss of intracellular potassium, which is known to occur in a potassium deficiency (Heppel, '39, '40; Hegnauer, '43). That a minimum amount of potassium is required by the rat is evidenced by the reversal of the beneficial effect of sodium to a toxic effect when the potassium content was sufficiently low (less than 0.09%).

It would appear, then, that the rapidity of production of a potassium-deficient state is dependent, in part, on the sodium content of the ration. This has been demonstrated by the work of Meyer et al. ('50), wherein the death rate and degree of heart lesions of potassium-deficient rats were dependent on the sodium content of the ration. The demonstration by Gillis ('48) that the potassium requirement of the chick is decreased by an increase in the phosphorus content of the ration is additional evidence for the fact that the requirements for minerals as well as other nutrients cannot be stated dogmatically without reference to the remaining constituents of the ration.

The comparatively rapid decrease of the potassium requirement, as compared to the sodium requirement, with an increase in age, does not appear surprising when the distribution of the two ions within the body is taken into account. The intracellular prominence of potassium and its close association with enzymatic reactions would suggest a partial regulation of its requirement by the rate of metabolic processes and growth in the body. The demonstrations by Mitchell and Carmann ('26) and Kibler and Brody ('42) that the basal metabolic rate of the rat decreases within the age limits studied here are in agreement with this hypothesis. The extracellular activity of sodium would tend to minimize the influence of the rate of metabolic processes on the requirement. Consequently, the sodium requirement would tend to be relatively constant over a similar time interval.

SUMMARY

The sodium and potassium requirements of the rat for growth have been investigated, with the following results:

The growth requirement of the rat for sodium was 0.05% and it was independent of the potassium content within limits of 0.25 to 1.0% potassium.

The potassium requirement was 0.18% in the presence of 0.1% sodium. A high level of sodium (1.0%) reduced the potassium requirement to 0.15%. An initial sparing action by sodium was exhibited provided a minimum amount of potassium was present in the ration. When the potassium was present at less than 0.09%, the initial sparing action of sodium not only disappeared but a potassium deficiency resulted.

It was found that the potassium requirement decreased progressively with increase in age during the 6-week growing period. In contrast, the sodium requirement remained relatively constant throughout the experimental period. One per cent potassium had a growth depressing effect. This was not observed in the case of sodium fed at a 1.0% level.

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THE ISOLEUCINE REQUIREMENT FOR THE GROWTH OF SWINE

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Previous communications from this laboratory have emphasized the importance of methionine (Bell et al., '50) and the level of lysine (Brinegar et al., '50) in rations for growing swine. Mertz et al. ('49) had previously demonstrated the need for lysine in rations for growing pigs. Reports by Beeson et al. ('48) and Shelton et al. ('49) show that tryptophan is also an indispensable dietary component of swine rations. The investigation described in the present report was undertaken to demonstrate the essential role of isoleucine in rations for growing pigs.

A comparison of the amino acid content of whole blood protein to that of whole egg protein (Block and Mitchell, '46–'47) indicates that blood is an excellent source of amino acids, with the exception of isoleucine and possibly methionine. The chick feeding experiments of Grau and Almquist ('44) showed that the poor quality of blood protein is due primarily to a deficiency in isoleucine.

This study was designed to determine the isoleucine requirement of weanling pigs by supplementing a ration containing blood flour fortified with methionine and with several levels of isoleucine.

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PROCEDURE

The basal diet and the amino acid composition of the protein in the basal diet, as determined microbiologically, are shown in table 1. Methionine was included in the diet since a comparison of the content of this amino acid in blood protein with that in proteins having higher nutritive values for

TABLE 1

Ingredients and amino acid composition of the basal diet

CONSTITUENTS		AMINO ACID COMPOSITION [6] GM PER 16 GM NITROGEN	
	%		
Blood flour [1]	23.68	Arginine	4.2
DL-methionine	0.40	Histidine	6.1
Starch	36.42	Lysine	8.9
Glucose (cerelose)	25.00	Tryptophan	1.3
Ruffex [2]	3.75	Tyrosine	2.9
Mineral salts [3]	5.00	Phenylalanine	6.8
Lard	4.75	Methionine [7]	3.0
Liver fraction "L" [4]	1.00	Threonine	4.4
Vitamins [5]	+	Leucine	9.6
		Isoleucine [8]	1.1
		Valine	8.3

[1] Wilson's spray-dried soluble blood flour.

[2] A commercial rice hull preparation by the Fisher Scientific Co.

[3] The following ingredients were included in the mineral mixture: $CaHPO_4 \cdot 2H_2O$, 65.5%; NaCl, 25.0%; KCL, 7.0%; MgO, 2.0% $FeSO_4 \cdot 7H_2O$, 0.4%; KI, 0.026%; $MnSO_4 \cdot 5H_2O$, 0.025%; $CuSO_4 \cdot 5H_2O$, 0.025%; $CoCl_2$, 0.012%; and $ZnCl_2$, 0.012%.

[4] Obtained from the Wilson Laboratories, Chicago, Ill.

[5] See Brinegar et al. ('50).

[6] Determined by microbiological techniques to be published in detail later.

[7] Includes addition of crystalline DL-methionine.

[8] Before supplementation with isoleucine.

the rat (Block and Mitchell, '46–'47) indicated that blood protein is possibly deficient in methionine.

Five levels of DL-isoleucine (with allo-isoleucine) were substituted for equivalent amounts of cerelose, making a total of 6 experimental diets ranging in protein content from 20.8% to 22.1% ($N \times 6.25$). The L-isoleucine content of the basal ration and of the isoleucine supplement was determined by

microbiological assay, using *Streptococcus faecalis* (9790).[2] The results of the experiments conducted by Smith and Greene ('48) showed that allo-isoleucine is biologically inactive for *Streptococcus faecalis.*

The growth response of weanling Yorkshire pigs, which were individually fed the experimental diets, was measured over a 4-week period following a preliminary adjustment period of at least 5 days. Daily intakes of air-dried feed were equalized weekly at approximately 5% of body weight. Nitrogen balance data were also obtained for some of the pigs for a 5-day period.

RESULTS AND DISCUSSION

It has been shown that only the natural isomer of isoleucine is utilized by the rat (Rose, '37), the mouse (Bauer and Berg, '43), the chick (Grau and Peterson, '46) and apparently the human (Rose, '49). On the assumption that the same may be true for pigs, the levels of total L-isoleucine in the various diets are shown in table 2, along with the amounts of DL-isoleucine (with allo-isoleucine) fed.

There were originally 5 pigs on each of the experimental diets; however, one pig on each of the diets containing 0.23% and 0.46% L-isoleucine failed to consume enough feed to permit significant growth. The daily feed consumption of the pig receiving 0.46% L-isoleucine was less than 3.65% of its body weight and it gained only 136 gm per day during the 4-week period. The daily feed consumption of the pig receiving 0.23% L-isoleucine was only 3.30% of its body weight for the first two weeks, during which time it lost weight. During the last two weeks it was fed the diet containing 0.81% L-isoleucine. The daily feed consumption of this pig increased to 4.12% of its body weight, while it gained 538 gm

[2] This method showed the isoleucine supplement to contain 38% L-isoleucine. The basal ration contained 0.23% L-isoleucine. The standard used for isoleucine was generously furnished by Dr. E. E. Howe of Merck and Co., Rahway, New Jersey. He indicated that, according to solubility analysis, the sample was more than 99% DL-isoleucine.

per day, with a feed efficiency of 663 gm gain in weight per kilogram of feed consumed. The average feed intakes of the remaining pigs were satisfactory and were similar enough to permit valid comparisons between levels of isoleucine.

The diets containing 0.46%, 0.58% and 0.70% L-isoleucine each produced larger gains, with correspondingly greater feed efficiency, than the diets containing less isoleucine (table 2). The gains and the efficiency of feed utilization of the pigs

TABLE 2

Average data for pigs receiving various levels of isoleucine in the diet containing blood flour

	SUPPLEMENTAL LEVEL OF DL-ISOLEUCINE, WITH ALLO-ISOLEUCINE (%)					
	None	0.615	0.923	1.230	1.538	1.845
Total L-isoleucine (%)	0.23	0.46	0.58	0.70	0.81	0.93
Number of pigs [1]	4	4	5	5	5	5
Initial weight (kg)	18.8	18.8	18.1	18.2	17.9	17.6
Daily feed intake (% of body weight)	4.54	4.36	4.48	4.42	4.45	4.48
Daily gain (gm)	332	523	578	630	574	598
Grams gained per kilogram of feed	312	456	493	548	499	515
Apparent digestibility of nitrogen (%) [2]	94.0	95.0	...	95.7	...	...
Apparently digested nitrogen retained (%) [2]	24.4	31.8	...	42.1	...	...

[1] One of the pigs receiving 0.46% L-isoleucine and two of the pigs receiving 0.7% L-isoleucine were on trial for only 21 days. The remaining pigs were on trial for 28 days.

[2] Determinations using three pigs on each of the diets containing 0.46% and 0.7% L-isoleucine.

receiving the diets containing 0.81% and 0.93% L-isoleucine were less than those of the animals receiving 0.7% L-isoleucine. Grau and Peterson ('46) noted that chicks receiving 2.0% DL-isoleucine gained at a slightly lower rate than chicks receiving only 1.0% DL-isoleucine. It is possible that the relatively high levels of isoleucine (2.08% total isoleucine at the highest level) in our swine diets actually had a growth depressing effect.

The data for the retention of the apparently digested nitrogen show that the more rapid growth rates of the pigs receiving supplements of isoleucine were related to increases in the efficiency of utilization of the dietary nitrogen. The pigs which received 0.7% L-isoleucine retained 73% more nitrogen and made 90% greater daily gains, with a 76% greater efficiency of feed utilization, than those which were fed the diet containing only 0.23% L-isoleucine.

Nitrogen balance data (Brinegar, '50) from weanling pigs fed a diet containing 9.6% of protein from blood flour supplemented with methionine and isoleucine showed that the nitrogen of the blood flour used in these studies was almost completely digested (99.6%), and that the protein of the properly supplemented blood flour had a biological value of 90. This high degree of utilization indicates that the L-isoleucine requirement of 0.7% of the diet should not be expected to increase if a different source of amino acids is used in the basal ration, and that this requirement can be considered as referring to digestible L-isoleucine.

There are other factors, however, which should be considered in applying the L-isoleucine requirement of 0.7% of the diet to actual feeding practice. It applies only to the size and stage of growth of the pigs used in these trials, and particularly to rations containing approximately 22% of protein. Studies on the lysine requirement of swine (Brinegar et al., '50) emphasize the importance of considering the level of dietary protein in evaluating an amino acid requirement for weanling pigs. The L-isoleucine requirement of 0.7% of the diet is equivalent to 3.2% of the protein in the diet.

SUMMARY

A basal diet containing approximately 22% protein and consisting of blood flour supplemented with methionine was used to determine the isoleucine requirement for early growth of swine. The effects of adding various levels of DL-isoleucine (with allo-isoleucine) to this diet for weanling pigs were stud-

ied over a 4-week period. The daily feed intakes of the pigs were controlled at approximately 4.5% of body weight.

The maximum response in growth and feed efficiency was obtained with the diet containing 0.7% L-isoleucine. This isoleucine requirement is equivalent to 3.2% of the dietary protein.

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OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1951 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1951.

ROBERT V. BOUCHER
Pennsylvania State College
State College, Pennsylvania

CHAIRMAN, NOMINATING COMMITTEE

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1951 Award of $1000.00 and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the Jury of Award may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1951. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1951. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

HAZEL K. STIEBELING
Bureau of Human Nutrition and Home Economics
U. S. Department of Agriculture
Washington, D. C.

CHAIRMAN, NOMINATING COMMITTEE

MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1951 Award of $1000.00 established by Mead Johnson and Company to promote researches dealing with the B-complex vitamins. The recipient of this Award will be chosen by a Jury of Award of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the spring of 1951.

The Award will be given to the laboratory or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year, January 1 to December 31, the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Jury of Award, nominations for this award for work published in 1950 must be in the hands of the Chairman of the Nominating Committee by January 1, 1951. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Jury of Award in its consideration of the nomination.

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Chairman, Nominating Committee

INDEX

ACETATE (vitamin A), vitamin A alcohol and vitamin A natural esters, relative utilization as affected by variations in dosage level, quantity and character of diluent and quantity of dietary fat, 525.
Ad libitum and force-feeding conditions, methionine deficiency under, 423.
Adrenal, mouse, effect of protein deficiency and other dietary factors on X zone of, 157.
Alcohol (vitamin A), vitamin A acetate and vitamin A natural esters, relative utilization as affected by variations in dosage level, quantity and character of diluent, and quantity of dietary fat, 525.
Alfalfa diets, high, or diet containing Quillaja saponin, effect of sterols on growth of chicks fed, 597.
American Institute of Nutrition
Borden award in nutrition, presentation, 1950, 150.
Committee on Biochemical Nomenclature, report of, 148.
— on Program Policy, report of, 145.
— on rules for awards, report of, 142.
Editor of Journal of Nutrition, report of, 1950, 140.
Editorial Board, 1950, 141.
Election of officers for 1950–51, 147.
Mead Johnson and Company 'B-complex' award, presentation, 1950, 149.
New members elected, 1950, 148.
Osborne and Mendel award, presentation, 1950, 150.
Proceedings, 14th annual meeting, 1950, 139.
Amino acid(s), excretion in normal and undernourished human subjects following a single infusion of an amino acid preparation, 71.
—— preparation, excretion of amino acids in normal and undernourished human subjects following a single infusion of an, 71.
—— supplementation of wheat with, in diet of chick, 565.
p-Aminobenzoic acid, pantothenic acid, biotin and inositol, studies on. Nutrition of mouse. VIII., 257.
ANDERSON, J. O., G. F. COMBS AND G. M. BRIGGS. Niacin-replacing value of L- and DL-tryptophan in chick diets as influenced by carbohydrate source, 463.
Animal protein factor concentrates in nutrition of mature fowl, 473.
Aqueous and oily media, vitamin A stores of sucklings following administration to dams of vitamin A in, 557.
Artificial enrichment of white rice as a solution to endemic beriberi. Report of field trials in Bataan, Philippines, 501.
Ascorbic acid metabolism (see Metabolism, ascorbic acid).
Award(s), Borden, in nutrition (see Borden award).
— Mead Johnson and Company 'B-complex' (see Mead Johnson and Company).
— Osborne and Mendel (see Osborne and Mendel award).
— rules governing, report of committee on (see American Institute of Nutrition).

B vitamins (see Vitamins, B).
BAMBA, M. D. See Salcedo, J., Jr., 501.
BANCROFT, R. W. See Geiger, E., 577.
BARROWS, L. See Chow, B. F., 405.
Bataan, Philippines, nutrition survey and tests in, 9.
—— report of field trials in. Artificial enrichment of white rice as solution to endemic beriberi, 501.
BAVETTA, L. A. See Deuel, H. J., Jr., 239.
BELL, J. M. See Farmer, F. A., 309.
BENUA, R. S. See Howard, E., 157.
Beriberi endemic, artificial enrichment of white rice as solution to. Report of field trials in Bataan, Philippines, 501.
BIELY, J. See March, B., 565.
Biography of C. Eijkman, 3.
Biotin, pantothenic acid, inositol and *p*-aminobenzoic acid, studies on. Nutrition of mouse. VIII., 257.
BLACK, A., K. H. MADDY AND R. W. SWIFT. The influence of low levels of protein on heat production, 415.
Blood, vitamin E levels in, vitamin A and carotene utilization and other chemical studies. Vitamin E in nutrition of cattle. II., 391.
Bone formation and growth, further studies on role of potassium in, 45.
Borden award in nutrition, 1951, announcement, 155, 626; presentation, 1950, 150.
BOYER, P. D. See Kachmar, J. F., 391.
BRATZLER, J. W., J. K. LOOSLI, V. N. KRUKOVSKY AND L. A. MAYNARD. Effect of the dietary level of tocopherols on their metabolism in swine, 59.
Brewers' yeast (see Yeast, brewers').
BRIGGS, G. M. See Anderson, J. O., 463.
BRINEGAR, M. J., J. K. LOOSLI, L. A. MAYNARD AND H. H. WILLIAMS. The isoleucine requirement for the growth of swine, 619.
— H. H. WILLIAMS, F. H. FERRIS, J. K. LOOSLI AND L. A. MAYNARD. The lysine requirement for the growth of swine, 129.
BURCH, H. B., J. SALCEDO, JR., E. O. CARRASCO, C. L. INTENGAN AND A. B. CALDWELL. Nutrition survey and tests in Bataan, Philippines, 9.
Butterfat replaced by margarine fat in Sherman diet B, growth and reproduction over 25 generations on, including a study of calcium metabolism, 239.

CALCIUM and iron, perspiration as factor influencing requirement for, 285.
— metabolism (see Metabolism, calcium).
CALDWELL, A. B. See Burch, H. B., 9.
Canned foods (see Foods, canned).
Carbohydrate, nitrogen-sparing effect of dietary, in its relation to the time factor. Experiments with repletion of protein-depleted adult rats, 577.
— source, niacin-replacing value of L- and DL-tryptophan in chick diets as influenced by, 463.
Carotene and vitamin A utilization, vitamin E levels in blood and other chemical studies. Vitamin E in nutrition of cattle. II., 391.
CARRASCO, E. O. See Burch, H. B., 9; Salcedo, J., Jr., 501.
CARRETERO, R. See Snyderman, S. E., 31.
Cattle, vitamin E in nutrition of. II., 391.
CHAN, G. S. See Salcedo, J., Jr., 501.
CHELDELIN, V. H. See King, T. E., 195.
Chemical studies, other, vitamin E levels in blood, vitamin A and carotene utilization and. Vitamin E in nutrition of cattle. II., 391.
Chick(s) diets, niacin-replacing value of L- and DL-tryptophan in, as influenced by carbohydrate source, 463.
— effects of dietary supplements on plasma tocopherol levels and vitamin E deficiency symptoms in, 359.
— fed high alfalfa diets or diet containing Quillaja saponin, effect of sterols on growth of, 597.

— growing, essential role of fatty acids in rations for, 319.
—— metabolism of polyunsaturated fatty acids in, 325.
— plasma xanthophyll levels and vitamin E deficiency symptoms in, 453.
— supplementation of wheat with amino acids in diet of, 565.
— tissues, vitamin B_{12} content of, as influenced by diet, 337.
— vitamin E deficiency in. I., 359; II., 453.
Children (selected Louisiana elementary school), hemoglobin concentrations, erythrocyte counts, and hematocrits of, 539.
CHITRE, R. G., J. N. WILLIAMS, JR. AND C. A. ELVEHJEM. Nutritive value of canned foods. XLII. A study of the protein value of young and mature peas (Pisum sativum), 207.
CHOW, B. F., L. BARROWS AND C. LANG. The microbiological activity of vitamin B_{12} in the urine of normal rats following the oral and subcutaneous administration of this vitamin, 405.
CHRISTENSEN, H. N. Peptide wastage consequent to the infusion of two protein hydrolysates, 189.
Citrate formation and growth in rats, influence of a pantothenic acid conjugate (PAC) upon, 195.
COMBS, G. F. See Anderson, J. O., 463.
CONCEPCION, I. See Salcedo, J., Jr., 501.
COUCH, J. R., AND O. OLCESE. The vitamin B_{12} content of chick tissues as influenced by the diet, 337.
—— B. G. SANDERS AND J. V. HALICK. Vitamin B_{12}. APF concentrates, dried whey, fish solubles and liver fraction "L" in the nutrition of the mature fowl, 473.
COWGILL, G. R. See Fenton, P. F., 257.
CRAMPTON, E. W. See Farmer, F. A., 309.
CSONKA, F. A. Nitrogen, methionine and cystine content of hen's eggs. Their distribution in the egg white and yolk, 443.
Cystine content of hen's eggs, distribution in egg white and yolk, 443.

DAMS, vitamin A stores of sucklings following administration to, of vitamin A in oily and aqueous media, 537.
DAY, P. L. See Dinning, J. S., 81.
Decay, experimental tooth, prevention by tube-feeding, 89.
Dehydroascorbic acid, utilization by human subjects, 297.
DE LEON, J. F. See Salcedo, J., Jr., 501.
DENTON, A. E., J. N. WILLIAMS, JR. AND C. A. ELVEHJEM. Methionine deficiency under ad libitum and force-feeding conditions, 423.
DEUEL, H. J., JR., S. M. GREENBERG, E. E. SAVAGE AND L. A BAVETTA. Studies on the comparative nutritive value of fats. XIII. Growth and reproduction over 25 generations on Sherman diet B where butterfat was replaced by margarine fat, including a study of calcium metabolism, 239.
— See Greenberg, S. M., 279.
Diet(s) B, Sherman, where butterfat was replaced by margarine fat, growth and reproduction over 25 generations on, including a study of calcium metabolism, 239.
— chick, niacin-replacing value of L- and DL-tryptophan in, as influenced by carbohydrate source, 463.
— containing Quillaja saponin, or high alfalfa diets, effect of sterols on growth of chicks fed, 597.
— deficient in reproductive factors, contributions of brewers' yeast to, 117.
— fecal excretion of lipids by rats as influenced by, 175.
— high alfalfa, or containing Quillaja saponin, effect of sterols on growth of chicks fed, 597.
—— fat, protective effect on immature rats fed thyroid, 279.
— of chick, supplementation of wheat with amino acids in, 565.
— vitamin B_{12} content of chick tissues as influenced by, 337.
Dietary carbohydrate, nitrogen-sparing effect of, in its relation to the time factor. Experiments with repletion of protein-depleted adult rats, 577.
— factor(s), other, and protein deficiency, effect on X zone of mouse adrenal, 157.
—— unknown, essential for human growth, observations on an, 31.
— fat, relative utilization of vitamin A alcohol, acetate and natural esters as affected by quantity of, 525.
— level of tocopherols, effect on their metabolism in swine, 59.
— supplements, effects on plasma tocopherol levels and vitamin E deficiency symptoms in chicks, 359.
Diluent, relative utilization of vitamin A alcohol, acetate and natural esters as affected by quantity and character of, 525.
Dinitrophenol toxicity in rat, comparative effects of B vitamins and liver on, 271.
DINNING, J. S., C. K. KEITH, J. T. PARSONS AND P. L. DAY. The influence of pteroylglutamic acid and vitamin B_{12} on the metabolism of pyridine-fed rats, 81.
Dosage level, relative utilization of vitamin A alcohol, acetate and natural esters as affected by variations in, 525.
Dried whey (See Whey, dried).

EASTERLING, L. See Sure, B., 221.
Egg(s) (hen's), nitrogen, methionine and cystine content of, distribution in egg white and yolk, 443.
— white and yolk; nitrogen, methionine and cystine content of hen's eggs and distribution in, 443.
EHMKE, D. A. See Todhunter, E. N., 297.
EIJKMAN, C. (August 11, 1858 – November 5, 1930), 3.
Elementary school children (selected Louisiana), hemoglobin concentrations, erythrocyte counts, and hematocrits of, 539.
ELLIS, L. N. See Spencer, A. J., Jr., 107.
ELVEHJEM, C. A. See Chitre, R. G., 207; Denton, A. E., 423; Tove, S. B., 433.
Endemic beriberi, artificial enrichment of white rice as solution to. Report of field trials in Bataan, Philippines, 501.
Enrichment, artificial, of white rice as solution to endemic beriberi. Report of field trials in Bataan, Philippines, 501.
ERSHOFF, B. H. Comparative effects of B vitamins and liver on dinitrophenol toxicity in the rat, 271.
Erythrocyte counts of selected Louisiana elementary school children, 539.
Esters (vitamin A natural), vitamin A alcohol and vitamin A acetate, relative utilization as affected by variations in dosage level, quantity and character of diluent, and quantity of dietary fat, 525.
Etching of teeth, effect of fluoride and grapefruit juice on, 107.
EVANS, E. R. See Johnston, F. A., 285.
Excretion, fecal (see Fecal excretion).
— of amino acids in normal and undernourished human subjects following a single infusion of an amino acid preparation, 71.
Experimental tooth decay, prevention by tube-feeding, 89.
Experiments with repletion of protein-depleted adult rats. Nitrogen-sparing effect of dietary carbohydrate in its relation to the time factor, 577.

FACTOR, unknown, with vitamin A activity distilled from lard, 375.
FARMER, F. A., B. C. MUTCH, J. M. BELL, L. D. WOOLSEY AND E. W. CRAMPTON. The vitamin E requirement of guinea pigs, 309.
Fat(s), comparative nutritive value of. XIII., 239.

— dietary, relative utilization of vitamin A alcohol, acetate and natural esters as affected by quantity of, 525.
— diets, high, protective effect on immature rats fed thyroid, 279.
— (margarine), growth and reproduction over 25 generations on Sherman diet B where butterfat was replaced by, including a study of calcium metabolism, 239.
Fatty acids, essential role in rations for growing chicks, 319.
—— polyunsaturated, metabolism in growing chicks, 325.
Fecal excretion of lipids by rats as influenced by diet, 175.
FENTON, P. F., G. R. COWGILL, M. A. STONE AND D. H. JUSTICE. The nutrition of the mouse. VIII. Studies on pantothenic acid, biotin, inositol and *p*-aminobenzoic acid, 257.
FERRIS, F. H. See Brinegar, M. J., 129.
Field trials in Bataan, Philippines, report of. Artificial enrichment of white rice as solution to endemic beriberi, 501.
Fish solubles in nutrition of mature fowl, 473.
Fluoride and grapefruit juice, effect on etching of teeth, 107.
Foods, canned, nutritive value of. XLII., 207.
Force-feeding and ad libitum conditions, methionine deficiency under, 423.
Fowl (mature), vitamin B_{12}, APF concentrates, dried whey, fish solubles and liver fraction "L" in nutrition of, 473.
Fraction "L" (liver) in nutrition of mature fowl, 473.

GEIGER, E., R. W. BANCROFT AND E. B. HAGERTY. The nitrogen-sparing effect of dietary carbohydrate in its relation to the time factor. Experiments with repletion of protein-depleted adult rats, 577.
Generations, 25, on Sherman diet B where butterfat was replaced by margarine fat, growth and reproduction over, including a study of calcium metabolism, 239.
GIBSON, B. See Reiser, R., 325.
GILLIS, M. B. Further studies on the role of potassium in growth and bone formation, 45.
GOLDHABER, P., L. ZACHARIAS AND V. E. KINSEY. Vitamin E deficiency in chicks. II. Plasma xanthophyll levels and vitamin E deficiency symptoms, 453.
— See Zacharias, L., 359.
Grapefruit juice and fluoride, effect on etching of teeth, 107.
GREENBERG, S. M., AND H. J. DEUEL, JR. The protective effect of high fat diets on immature rats fed thyroid, 279.
— See Deuel, H. J., Jr., 239.
Growing chicks (see Chicks, growing).
Growth and bone formation, further studies on role of potassium in, 45.
— and citrate formation in rats, influence of a pantothenic acid conjugate (PAC) upon, 195.
— and reproduction over 25 generations on Sherman diet B where butterfat was replaced by margarine fat, including a study of calcium metabolism, 239.
— ascorbic acid metabolism in guinea pigs in relation to, 347.
— human, observations on an unknown dietary factor essential for, 31.
— of chicks fed high alfalfa diets or diet containing Quillaja saponin, effect of sterols on, 597.
— of rat, sodium and potassium requirements for, 609.
— of swine, isoleucine requirement for, 619; lysine requirement for, 129.
GRUNERT, R. R., J. H. MEYER AND P. H. PHILLIPS The sodium and potassium requirements of the rat for growth, 609.
Guinea pigs, ascorbic acid metabolism in, in relation to growth, 347.
—— vitamin E requirement of, 309.
GULLICKSON, T. W. See Kachmar, J. F., 391.
GUY, L. P. See Snyderman, S. E., 31.

HAGERTY, E. B. See Geiger, E., 577.
HALICK, J. V. See Couch, J. R., 473.
HALVORSON, H. O., AND M. O. SCHULTZE. Nutritional value of plant materials. III. Acute uremia of newborn rats from mothers fed plant rations, 227.
HARROUN, J. E. See Levey, S., 71.
Hay (soybean) feeding, sterility in rabbit associated with, 487.
Heat production, influence of low levels of protein on, 415.
Hematocrits of selected Louisiana elementary school children, 539.
Hemoglobin concentrations of selected Louisiana elementary school children, 539.
Hen's eggs (see Eggs, hen's).
Hog intestinal mucosa, unidentified factor present in, required by mink, 433.
HOGANSON, E. D. See Levey, S., 71.
HOLT, L. E., JR. See Snyderman, S. E., 31.
HOWARD, E., AND R. S. BENUA. The effect of protein deficiency and other dietary factors on the X zone of the mouse adrenal, 157.
Human growth, observations on an unknown dietary factor essential for, 31.
— subjects (see Man).
Hydrolysates, protein, peptide wastage consequent to the infusion of two, 189.

INFUSION of two protein hydrolysates, peptide wastage consequent to, 189.
— single, of an amino acid preparation, excretion of amino acids in normal and undernourished human subjects following, 71.
Inositol, pantothenic acid, biotin and *p*-aminobenzoic acid, studies on. Nutrition of mouse. VIII., 257.
INTENGAN, C. L. See Burch, H. B., 9.
Intestinal mucosa (hog), unidentified factor present in, required by mink, 433.
Iron and calcium, perspiration as factor influencing requirement for, 285.
Isoleucine requirement for growth of swine, 619.

JANSEN, B. C. P. C. Eijkman (August 11, 1858 – November 15, 1930), 3.
JOHNSON, R. E. See Kaunitz, H., 375.
JOHNSTON, F. A., T. MCMILLAN AND E. R. EVANS. Perspiration as a factor influencing the requirement for calcium and iron, 285.
JOSE, F. R. See Salcedo, J., Jr., 501.
Juice, grapefruit (see Grapefruit juice).
JUSTICE, D. H. See Fenton, P. F., 257.

KACHMAR, J. F., P. D. BOYER, T. W. GULLICKSON, E. LIEBE AND R. M. PORTER. Vitamin E in the nutrition of cattle. II. Vitamin E levels in the blood, vitamin A and carotene utilization, and other chemical studies, 391.
KAUNITZ, H., AND C. A. SLANETZ. An unknown factor with vitamin A activity distilled from lard, 375.
KEITH, C. K. See Dinning, J. S., 81.
KENDALL, K. A., G. W. SALISBURY AND N. L. VANDEMARK. Sterility in the rabbit associated with soybean hay feeding, 487.
KETRON, K. C. See Snyderman, S. E., 31.
KING, T. E., F. M. STRONG AND V. H. CHELDELIN. Pantothenic studies. X. The influence of a pantothenic acid conjugate (PAC) upon growth and citrate formation in rats, 195.
KINSEY, V. E. See Goldhaber, P., 453; Zacharias, L., 359.
KITE, O. W., J. H. SHAW AND R. F. SOGNNAES. The prevention of experimental tooth decay by tube-feeding, 89.
KRUKOVSKY, V. N. See Bratzler, J. W., 59.

LALOR, R. J. See Tove, S. B., 433.
LANG, C. See Chow, B. F., 405.
Lard, unknown factor with vitamin A activity distilled from, 375.
LEVEY, S., E. D. HOGANSON, J. E. HARROUN AND C. J. SMYTH. The excretion of amino acids in normal and undernourished human subjects following a single infusion of an amino acid preparation, 71.
LIEBE, E. See Kachmar, J. F., 391.
Lipids, fecal excretion by rats, as influenced by diet, 175.
Liver and B vitamins, comparative effects on dinitrophenol toxicity in rat, 271.
— fraction "L" in nutrition of mature fowl, 473.
LOOSLI, J. K. See Bratzler, J. W., 59; Brinegar, M. J., 129, 619.
Louisiana elementary school children (selected), hemoglobin concentrations, erythrocyte counts and hematocrits of, 539.
Lysine requirement for growth of swine, 129.

MADDY, K. H. See Black, A., 415.
Man, normal and undernourished, excretion of amino acids in, following a single infusion of an amino acid preparation, 71.
— utilization of dehydroascorbic acid by, 297.
MARCH, B., J. BIELY AND J. TONZETICH. Supplementation of wheat with amino acids in the diet of the chick, 565.
Margarine fat, growth and reproduction over 25 generations on Sherman diet B, where butterfat was replaced by, including a study of calcium metabolism, 239.
Mature fowl (see Fowl, mature).
— peas (Pisum sativum), protein value of, 207.
MAYNARD, L. A. See Bratzler, J. W., 59; Brinegar, M. J., 129, 619.
MCBEE, M., D. S. MOSCHETTE AND C. TUCKER. The hemoglobin concentrations, erythrocyte counts, and hematocrits of selected Louisiana elementary school children, 539.
MCMILLAN, T. See Johnston, F. A., 285; Todhunter, E. N., 297.
Mead Johnson and Company 'B-complex' award, 1950, presentation, 149; 1951, announcement, 153, 627.
Metabolism, ascorbic acid, in guinea pigs in relation to growth, 347.
— (calcium), growth and reproduction over 25 generations on Sherman diet B where butterfat was replaced by margarine fat, including a study of, 239.
— of polyunsaturated fatty acids in growing chicks, 325.
— of pyridine-fed rats, influence of pteroylglutamic acid and vitamin B_{12} on, 81.
— of tocopherols in swine, effect of their dietary level on, 59.
Methionine content of hen's eggs, distribution in egg white and yolk, 443.
— deficiency under ad libitum and force-feeding conditions, 423.
MEYER, J. H. See Grunert, R. R., 609.
Microbiological activity of vitamin B_{12} in urine of normal rats following oral and subcutaneous administration of this vitamin, 405.
Mink, unidentified factor present in hog intestinal mucosa required by, 433.
MOSCHETTE, D. S. See McBee, M., 539.
Mothers (rat) fed plant rations, acute uremia of newborn from. Nutritional value of plant materials. III., 227.
Mouse adrenal, effect of protein deficiency and other dietary factors on X zone of, 157.
— nutrition of. VIII., 257.
Mucosa (hog intestinal), unidentified factor present in, required by mink, 433.
MUTCH, B. S. See Farmer, F. A., 309.

NELL, R. E., AND P. H. PHILLIPS. Contributions of brewers' yeast to a diet deficient in reproductive factors, 117.
NEMIR, R. L. See Snyderman, S. E., 31.
Newborn rats (see Rats, newborn).
Niacin-replacing value of L- and DL-tryptophan in chick diets as influenced by carbohydrate source, 463.
Nitrogen content of hen's eggs, distribution in egg white and yolk, 443.
— sparing effect of dietary carbohydrate in its relation to the time factor. Experiments with repletion of protein-depleted adult rats, 577.
Normal human subjects (see Man, normal).
— rats (see Rats, normal).
Nutrition of cattle, vitamin E in. II., 391.
— of mature fowl; vitamin B_{12}, APF concentrates, dried whey, fish solubles and liver fraction "L" in, 473.
— of mouse. VIII. Studies on pantothenic acid, biotin, inositol and *p*-aminobenzoic acid, 257.
— survey and tests in Bataan, Philippines, 9.
Nutritional value of plant materials. III. Acute uremia of newborn rats from mothers fed plant rations, 227; V. Reproduction of rats fed purified rations containing soybean protein, 587.
Nutritive value, comparative, of fats. XIII. Growth and reproduction over 25 generations on Sherman diet B where butterfat was replaced by margarine fat, including a study of calcium metabolism, 239.
— — of canned foods. XLII. A study of the protein value of young and mature peas (Pisum sativum), 207.

OILY and aqueous media, vitamin A stores of sucklings following administration to dams of vitamin A in, 557.
OLCESE, O. See Couch, J. R., 337, 473.
OLIVEROS, S. B. See Salcedo, J., Jr., 501.
Oral and subcutaneous administration of vitamin B_{12}, microbiological activity of this vitamin in urine of normal rats following, 405.
Osborne and Mendel award, 1950, presentation, 150; 1951, announcement, 154, 625.

PAC (pantothenic acid conjugate), influence upon growth and citrate formation in rats, 195.
Pantothenic acid, biotin, inositol and *p*-aminobenzoic acid, studies on. Nutrition of mouse. VIII., 257.
— — conjugate (PAC), influence upon growth and citrate formation in rats, 195.
— studies. X. Influence of a pantothenic acid conjugate (PAC) upon growth and citrate formation in rats, 195.
PARSONS, J. T. See Dinning, J. S., 81.
PASCUAL, C. R. See Salcedo, J., Jr., 501.
Peas (Pisum sativum), young and mature, protein value of, 207.
Peptide wastage consequent to the infusion of two protein hydrolysates, 189.
Perspiration as factor influencing requirement for calcium and iron, 285.
PETERSON, D. W. Effect of sterols on the growth of chicks fed high alfalfa diets or a diet containing Quillaja saponin, 597.
Philippines (Bataan), nutrition survey and tests in, 9.
— — report of field trials in. Artificial enrichment of white rice as solution to endemic beriberi, 501.
PHILLIPS, P. H. See Grunert, R. R., 609; Nell, R. E., 117.
Pigs, guinea (see Guinea pigs).
Plant materials, nutritional value of. III., 227; V., 587.
— rations, acute uremia of newborn rats from mothers fed. Nutritional value of plant materials. III., 227.

Plasma tocopherol levels and vitamin E deficiency symptoms in chicks, effects of dietary supplements on, 359.
— xanthophyll levels and vitamin E deficiency symptoms in chicks, 453.
Polyunsaturated fatty acids, metabolism in growing chicks, 325.
PORTER, R. M. See Kachmar, J. F., 391.
Potassium and sodium requirements of rat for growth, 609.
— further studies on role in growth and bone formation, 45.
Proceedings, American Institute of Nutrition (see American Institute of Nutrition).
Protective action of vitamin B_{12} against toxicity of DL-thyroxine, 221.
Protein deficiency and other dietary factors, effect on X zone of mouse adrenal, 157.
— depleted adult rats, experiments with repletion of. Nitrogen-sparing effect of dietary carbohydrate in its relation to the time factor, 577.
— factor, animal (see Animal protein factor).
— hydrolysates, peptide wastage consequent to the infusion of two, 189.
— influence of low levels on heat production, 415.
— soybean, reproduction of rats fed purified rations containing. Nutritional value of plant materials. V., 587.
— value of young and mature peas (Pisum sativum), 207.
Pteroylglutamic acid and vitamin B_{12}, influence on metabolism of pyridine-fed rats, 81.
Purified rations (see Rations, purified).
Pyridine-fed rats, influence of pteroylglutamic acid and vitamin B_{12} on metabolism of, 81.

QUILLAJA saponin, effect of sterols on growth of chicks fed high alfalfa diets or diet containing, 597.

RABBIT, sterility in, associated with soybean hay feeding, 487.
Rat(s), comparative effects of B vitamins and liver on dinitrophenol toxicity in, 271.
— fecal excretion of lipids by, as influenced by diet, 175.
— fed purified rations containing soybean protein, reproduction of. Nutritional value of plant materials. V., 487.
— immature, fed thyroid, protective effect of high fat diets on, 279.
— influence of a pantothenic acid conjugate (PAC) upon growth and citrate formation in, 195.
— newborn, from mothers fed plant rations, acute uremia of. Nutritional value of plant materials. III., 227.
— normal, microbiological activity of vitamin B_{12} in urine of, following oral and subcutaneous administration of this vitamin, 405.
— protein-depleted adult, experiments with repletion of. Nitrogen-sparing effect of dietary carbohydrate in its relation to the time factor, 577.
— pyridine-fed, influence of pteroylglutamic acid and vitamin B_{12} on metabolism of, 81.
— sodium and potassium requirements for growth, 609.
Rations for growing chicks, essential role of fatty acids in, 319.
— plant, acute uremia of newborn rats from mothers fed. Nutritional value of plant materials. III., 227.
— purified, containing soybean protein, reproduction of rats fed. Nutritional value of plant materials. V., 587.
REID, M. E. Ascorbic acid metabolism in guinea pigs in relation to growth, 347.
REISER, R. The essential role of fatty acids in rations for growing chicks, 319; the metabolism of polyunsaturated fatty acids in growing chicks, 325.
Repletion of protein-depleted adult rats, experiments with. Nitrogen-sparing effect of dietary carbohydrate in its relation to the time factor, 577.
Reproduction and growth over 25 generations on Sherman diet B where butterfat was replaced by margarine fat, including a study of calcium metabolism, 239.
— of rats fed purified rations containing soybean protein. Nutritional value of plant materials. V., 587.
Reproductive factors, contributions of brewers' yeast to diet deficient in, 117.
Rice, white, artificial enrichment of, as solution to endemic beriberi. Report of field trials in Bataan, Philippines, 501.
ROSENBERG, A. A. See Sobel, A. E., 557.

SALCEDO, J., JR., M. D. BAMBA, E. O. CARRASCO, G. S. CHAN, I. CONCEPCION, F. R. JOSE, J. F. DE LEON, S. B. OLIVEROS, C. R. PASCUAL, L. C. SANTIAGO AND R. C. VALENZUELA. Artificial enrichment of white rice as a solution to endemic beriberi. Report of field trials in Bataan, Philippines, 501.
— See Burch, H. B., 9.
SALISBURY, G. W. See Kendall, K. A., 487.
SANDERS, B. G. See Couch, J. R., 473.
SANTIAGO, L. C. See Salcedo, J., Jr., 501.
Saponin, Quillaja, effect of sterols on growth of chicks fed high alfalfa diets or diet containing, 597.
SAVAGE, E. E. See Deuel, H. J., Jr., 239.
School children (selected Louisiana elementary), hemoglobin concentrations, erythrocyte counts, and hematocrits of, 539.
SCHULTZE, M. O. Nutritional value of plant materials. V. Reproduction of rats fed purified rations containing soybean protein, 587.
— See Halvorson, H. O., 227.
SCHULZ, J. A., AND B. H. THOMAS. The fecal excretion of lipids by rats as influenced by the diet, 175.
SEVIGNE, F. J. See Week, E. F., 525.
SHAW, J. H. See Kite, O. W., 89.
Sherman diet B where butterfat was replaced by margarine fat, growth and reproduction over 25 generations on, including a study of calcium metabolism, 239.
SLANETZ, C. A. See Kaunitz, H., 375.
SMYTH, C. J. See Levey, S., 71.
SNYDERMAN, S. E., L. E. HOLT, JR., R. L. NEMIR, L. P. GUY, R. CARRETERO AND K. C. KETRON. Observations on an unknown dietary factor essential for human growth, 31.
SOBEL, A. E., AND A. A. ROSENBERG. Vitamin A stores of sucklings following administration to the dams of vitamin A in oily and aqueous media, 557.
Sodium and potassium requirements of rat for growth, 609.
SOGNNAES, R. F. See Kite, O. W., 89.
Solubles, fish (see Fish solubles).
Soybean hay feeding, sterility in rabbit associated with, 487.
— protein, reproduction of rats fed purified rations containing. Nutritional value of plant materials. V., 587.
SPENCER, A. J. JR., AND L. N. ELLIS. The effect of fluoride and grapefruit juice on the etching of teeth, 107.
Sterility in rabbit associated with soybean hay feeding, 487.
Sterols, effect on growth of chicks fed high alfalfa diets or diet containing Quillaja saponin, 597.
STONE, M. A. See Fenton, P. F., 257.
Stores (vitamin A) of sucklings following administration to dams of vitamin A in oily and aqueous media, 557.
STRONG, F. M. See King, T. E., 195.
Subcutaneous and oral administration of vitamin B_{12}, microbiological activity of this vitamin in urine of normal rats following, 405.

Sucklings, vitamin A stores of, following administration to dams of vitamin A in oily and aqueous media, 557.
SURE, B., AND L. EASTERLING. The protective action of vitamin B_{12} against the toxicity of DL-thyroxine, 221.
Survey and tests, nutrition, in Bataan, Philippines, 9.
SWIFT, R. W. See Black, A., 415.
Swine, effect of dietary level of tocopherols on their metabolism in, 59.
— isoleucine requirement for growth of, 619.
— lysine requirement for growth of, 129.

TEETH, effect of fluoride and grapefruit juice on etching of, 107.
Tests and survey, nutrition, in Bataan, Philippines, 9.
THOMAS, B. H. See Schulz, J. A., 175.
Thyroid, protective effect of high fat diets on immature rats fed, 279.
Thyroxine (DL-), protective action of vitamin B_{12} against toxicity of, 221.
Time factor, nitrogen-sparing effect of dietary carbohydrate in its relation to. Experiments with repletion of protein-depleted adult rats, 577.
Tissues, chick, vitamin B_{12} content of, as influenced by diet, 337.
Tocopherol(s), effect of dietary level of, on their metabolism in swine, 59.
— (plasma) levels and vitamin E deficiency symptoms in chicks, effects of dietary supplements on, 359.
TODHUNTER, E. N., T. MCMILLAN AND D. A. EHMKE. Utilization of dehydroascorbic acid by human subjects, 297.
TONZETICH, J. See March, B., 565.
Tooth decay, prevention of experimental, by tube-feeding, 89.
TOVE, S. B., R. J. LALOR AND C. A. ELVEHJEM. An unidentified factor present in hog intestinal mucosa required by the mink, 433.
Toxicity, dinitrophenol, in rat, comparative effects of B vitamins and liver on, 271.
— of DL-thyroxine, protective action of vitamin B_{12} against, 221.
Tryptophan (L- and DL-), niacin-replacing value in chick diets as influenced by carbohydrate source, 463.
Tube-feeding, prevention of experimental tooth decay by, 89.
TUCKER, C. See McBee, M., 539.

UNDERNOURISHED and normal human subjects, excretion of amino acids in, following a single infusion of an amino acid preparation, 71.
Uremia, acute, of newborn rats from mothers fed plant rations. Nutritional value of plant materials. III., 227.
Urine of normal rats, microbiological activity of vitamin B_{12} in, following oral and subcutaneous administration of this vitamin, 405.

VALENZUELA, R. C. See Salcedo, J., Jr., 501.
VANDEMARK, N. L. See Kendall, K. A., 487.
Vitamin(s) A activity, unknown factor with, distilled from lard, 375.
—— alcohol, acetate and natural esters, relative utilization as affected by variations in dosage level, quantity and character of diluent, and quantity of dietary fat, 525.
—— and carotene utilization, vitamin E levels in blood, and other chemical studies. Vitamin E in nutrition of cattle. II., 391.
—— in oily and aqueous media, vitamin A stores of sucklings following administration to dams of, 557.
—— stores of sucklings following administration to dams of vitamin A in oily and aqueous media, 557.
—— utilization studies. IV. Relative utilization of vitamin A alcohol, vitamin A acetate and vitamin A natural esters as affected by variations in dosage level, quantity and character of diluent, and quantity of dietary fat, 525.
— B and liver, comparative effects on dinitrophenol toxicity in rat, 271.
— B_{12} and peteroylglutamic acid, influence on metabolism of pyridine-fed rats, 81.
—— APF concentrates, dried whey, fish solubles and liver fraction "L" in nutrition of mature fowl, 473.
—— content of chick tissues as influenced by diet, 337.
—— microbiological activity in urine of normal rats following oral and subcutaneous administration, 405.
—— protective action against toxicity of DL-thyroxine, 221.
— E deficiency in chicks. I. Effects of dietary supplements on plasma tocopherol levels and vitamin E deficiency symptoms, 359; II. Plasma xanthophyll levels and vitamin E deficiency symptoms, 453.
—— symptoms and plasma tocopherol levels in chicks, effects of dietary supplements on, 359.
—— —— —— —— xanthophyll levels in chicks, 453.
—— in nutrition of cattle. II. Vitamin E levels in blood, vitamin A and carotene utilization, and other chemical studies, 391.
—— levels in blood, vitamin A and carotene utilization, and other chemical studies. Vitamin E in nutrition of cattle. II., 309.
—— requirement of guinea pigs, 309.

WASTAGE, peptide, consequent to the infusion of two protein hydrolysates, 189.
WEEK, E. F., AND F. J. SEVIGNE. Vitamin A utilization studies. IV. Relative utilization of vitamin A alcohol, vitamin A acetate and vitamin A natural esters as affected by variations in dosage level, quantity and character of diluent, and quantity of dietary fat, 525.
Wheat, supplementation with amino acids in diet of chick, 565.
Whey, dried, in nutrition of mature fowl, 473.
White, egg (see Egg white).
— rice (see Rice, white).
WILLIAMS, H. H. See Brinegar, M. J., 129, 619.
WILLIAMS, J. N., JR. See Chitre, R. G., 207; Denton, A. E., 423.
WOOLSEY, L. D. See Farmer, F. A., 309.

X zone of mouse adrenal, effect of protein deficiency and other dietary factors on, 157.
Xanthophyll (plasma) levels and vitamin E deficiency symptoms in chicks, 453.

YEAST, brewers', contributions to diet deficient in reproductive factors, 117.
Yolk (egg), nitrogen, methionine and cystine content of hen's eggs and distribution in egg white and, 443.
Young peas (Pisum sativum), protein value of, 207.

ZACHARIAS, L., P. GOLDHABER AND V. E. KINSEY. Vitamin E deficiency in chicks. I. The effects of dietary supplements on plasma tocopherol levels and vitamin E deficiency symptoms, 359.
— See Goldhaber, P., 453.
Zone (X) of mouse adrenal, effect of protein deficiency and other dietary factors on, 157.